APPENDIX A. WEIGHT-OF-EVIDENCE NARRATIVES

This appendix contains several general illustrations of weight-of-evidence narratives.

NARRATIVE #1
Substance #1
CAS# XXX

CANCER HAZARD SUMMARY

Substance 1 is *likely to be carcinogenic to humans by all routes of exposure*. The weight of evidence of human carcinogenicity of Substance 1 is based on (a) findings of carcinogenicity in rats and mice of both sexes by oral and inhalation exposures; (b) its similarity in structure to other chlorinated organics that are known to cause liver and kidney damage, and liver and kidney tumors in rats and mice; (c) suggestive evidence of a possible association between Substance 1 exposure of workers in the laundry and dry cleaning industries and increased cancer risk in a number of organ systems; and (d) human and animal data indicating that Substance 1 is absorbed by all routes of exposure.

In comparison with other agents designated as likely carcinogens, the overall weight of evidence for Substance 1 places it at the *low end* of the grouping. This is because one cannot attribute observed excess cancer risk in exposed workers solely to Substance 1. Moreover, there is considerable scientific uncertainty about the human significance and relevance of certain rodent tumors associated with exposure to Substance 1 and other chlorinated organics, but insufficient evidence about mode of action. Hence, the human relevance of the animal evidence of carcinogenicity relies on a default assumption.

There is no clear evidence about the mode of action for each tumor type induced in rats and mice. Available evidence suggests that Substance 1 induces cancer mainly by promoting cell growth rather than via direct mutagenic action, although a mutagenic mode of action for rat kidney tumors cannot be ruled out. The dos-response assessment should, therefore, adopt *both default approaches, nonlinear and linear*. It is recognized that the latter approach likely overestimates risk at low doses if the mode of action is primarily growth promoting. This approach, however, may be useful for screening analyses.

SUPPORTING INFORMATION

Human Data

A number of epidemiologic studies of dry cleaning and laundry workers have reported elevated incidences of lung, cervix, esophagus, kidney, blood, and lymphoid cancers. Many of these studies are confounded by coexposure to other petroleum solvents, making them limited for determining whether the observed increased cancer risks are causally related to Substance 1. The only investigation of dry cleaning workers with no known exposure to other chemicals did not evaluate other confounding factors such as smoking, alcohol consumption, and low socioeconomic status to exclude the possible contribution of these factors to cancer risks.

Animal Data

The carcinogenic potential of Substance 1 has been adequately investigated in two chronic studies in two rodent species, the first study by gavage and the second study by inhalation. Substance 1 is carcinogenic in the liver in both sexes of mice when tested by either route of exposure. It causes marginally increased incidences of mononuclear cell leukemia (MCL) in both sexes of rats and low incidences of a rare kidney tumor in male rats by inhalation. No increases in tumor incidence were found in rats treated with Substance 1 by gavage. This rat study was considered limited because of high mortality of the animals.

Although Substance 1 causes increased incidences of tumors at multiple sites in two rodent species, controversy surrounds each of the tumor endpoints concerning their relevance and/or significance to humans (see discussion under Mode of Action).

Other Key Data

Substance 1 is a member of a class of chlorinated organics that often cause liver and kidney toxicity and carcinogenesis in rodents. Like many chlorinated hydrocarbons, Substance 1 itself has tested negative in a battery of standard genotoxicity tests using bacterial and mammalian cell systems, including human lymphocytes and fibroblast cells. There is evidence, however, that a minor metabolite generated by an enzyme found in rat kidney tissue is mutagenic. This kidney metabolite has been hypothesized to be related to the development of kidney tumors in the male rat. This metabolic pathway appears to be operative in the human kidney.

Human data indicate that Substance 1 is readily absorbed via inhalation, but to a much lesser extent by skin contact. Animal data show that Substance 1 is absorbed well by the oral route.

MODE OF ACTION

The mechanisms of Substance 1-induced mouse liver tumors are not completely understood. One mechanism has been hypothesized to be mediated by a genotoxic epoxide metabolite generated by enzymes found in the mouse liver, but there is a lack of direct evidence in support of this mechanism. A more plausible mechanism that still needs to be further defined is related to liver peroxisomal proliferation and toxicity by TCA (trichloroacetic acid), a major metabolite of Substance 1. However, there are no definitive data indicating that TCA induces peroxisomal proliferation in humans.

The mechanisms by which Substance 1 induces kidney tumors in male rats are even less well understood. The rat kidney response may be related to either kidney toxicity or the activity of a mutagenic metabolite of the parent compound.

The human relevance of Substance 1-induced MCL in rats is unclear. The biological significance of marginally increased incidences of MCL has been questioned by some, since this tumor occurs spontaneously in the tested rat strain at very high background rates. On the other hand, it has been considered by others to be a true finding because there was a decreased time to onset of the disease and the disease was more severe in treated as compared with untreated control animals. The exact mechanism by which Substance 1 increases incidence of MCL in rats is not known.

Overall, there is not enough evidence to justify high confidence in a conclusion about any single mode of action; it would appear that more than a single mode operates in different rodent tissues. The apparent lack of mutagenicity of Substance 1 itself and its general growth-promoting effect on high-background tumors, as well as its toxicity toward mouse liver and rat kidney tissue, support the view that its predominant mode of action is cell growth promoting rather than mutagenic. A mutagenic contribution to the renal carcinogenicity due to a metabolite cannot be entirely ruled out.

NARRATIVE #2

Substance #2

CAS# XXX

CANCER HAZARD SUMMARY

There is *suggestive* evidence for carcinogenicity of Substance 2, but it is not sufficient for assessment of human carcinogenic potential.

The evidence on carcinogenicity consists of (a) data from an oral animal study showing a response only at the highest dose in female rats, with no response in males, and (b) the fact that

other low-molecular-weight chemicals in this class have shown tumorigenicity in the respiratory tract after inhalation. The one study of Substance 2 effects by the inhalation route was not adequately performed. The available evidence is too limited to describe human carcinogenicity potential or support dose-response assessment.

SUPPORTING INFORMATION

Human Data

An elevated incidence of cancer was reported in a cohort of workers in a chemical plant who were exposed to a mixture of chemicals, including Substance 2 as a minor component. The study is considered inadequate because of the small size of the cohort studied and the lack of adequate exposure data.

Animal Data

In a long-term drinking water study in rats, an increased incidence of adrenal cortical adenomas was found in the highest dosed females. No other significant finding was made. The oral rat study was well conducted by a standard protocol. In a 1-year study in hamsters at one inhalation dose, no tumors were seen. This study was inadequate because of high mortality and consequent short duration. The chemical is very irritating and is a respiratory toxicant in mammals. The animal data are too limited for conclusions to be drawn.

Structural Analogue Data

Substance 2's structural analogues, formaldehyde and acetaldehyde, both have carcinogenic effects on the rat respiratory tract.

Other Key Data

The weight of results of mutagenicity tests in bacteria, fungi, fruit flies, and mice leads to an overall conclusion of not mutagenic; Substance 2 is lethal to bacteria to a degree that makes testing difficult and test results difficult to interpret. The chemical is readily absorbed by all routes.

MODE OF ACTION

Data are not sufficient to judge whether there is a carcinogenic mode of action.

NARRATIVE #3

Substance #3

CAS# XXX

CANCER HAZARD SUMMARY

Substance 3 is *carcinogenic to humans by all routes of exposure*. Although several studies in workers fall short of establishing causality, when considered together, suggest an elevated risk of lung cancer after long-term exposure to Substance 3. More importantly, animal cancer bioassay studies and mechanistic studies in both animals and exposed humans have provided strong consistent results that support a level of concern equal to having conclusive epidemiologic evidence. The weight of evidence of human carcinogenicity of Substance 3 is based on (a) consistent evidence of carcinogenicity at multiple sites in both sexes of rats and mice by oral and inhalation exposure; (b) epidemiologic evidence suggestive of a possible association between exposure of industrial workers to Substance 3 and elevated risk of lung cancer, which is the tumor type consistently found in different test species and with different routes of administration; (c) mutagenic effects in numerous *in vivo* and *in vitro* test systems, which are similar to those found in humans; (d) a similar profile of p53 mutations in transgenic rodent and human lung tumor tissue; (e) membership in a class of DNA-reactive compounds that are regularly observed to cause carcinogenic and mutagenic effects in animals. Due to its ready absorption by all routes of exposure and rapid distribution throughout the body, Substance 3 is expected to pose a risk by all routes of exposure. The strong evidence of a mutagenic mode of action supports dose-response assessment that assumes *linearity* of the relationship.

SUPPORTING INFORMATION

Human Data

Elevated risks of lung cancer different than that associated with smoking have been reported in exposed workers in several studies. The interpretation of the studies separately is complicated by the lack of consistency in dose-response, latency period, and average age of appearance exposures, as well as by exposure to other agents. So, there is no single study that demonstrates that Substance 3 caused the effects. Nevertheless, several of the studies together are considered suggestive of Substance 3 carcinogenicity because they consistently show cancer elevation in the same tissue. Biomonitoring studies of exposed workers find DNA damage in blood lymphocytes and the degree of DNA damage correlates with the level and duration of Substance 3 exposure. More importantly, a mechanistic linkage is found for humans by observation of a similar profile of mutation in the p53 gene from the lung tumor tissue of the p53

transgenic mouse and exposed workers. This mutation spectra is consistent with the type of predominant DNA adducts induced by Substance 3. This evidence provides strong support to the positive suggestion from the worker cancer studies.

Animal Data

Substance 3 causes cancer in multiple tissue sites in rats and mice of both sexes by oral and inhalation exposure. In particular, there is a consistent trend of a similar tumor site found in the human studies, namely, an elevated incidence of lung tumor in different species/sexes and by different routes of exposure. The database is more extensive than usual and the studies are of good quality. The observation of multisite, multispecies carcinogenic activity by an agent is considered to be very strong evidence and is often the case with highly mutagenic agents. There are also strong evidence in many studies showing that Substance 3 is mutagenic across different phylogenetic levels including rodents, as well as in peripheral cells of exposed humans--a property that is very highly correlated with carcinogenicity. Further strengthening the concern for human cancer risk is a similar p53 mutation spectra observed in lung tumor tissue from the p53 transgenic mouse and human cancer biopsies. In humans, a large number of the cases had a mutation in p53 with a predominance of GC to AT transitions. The mutation spectra of Substance 3 associated lung tumors differed from patterns reported for sporadic and smoking related tumors.

Structural Analogue Data

SAR analysis indicates that Substance 3 is a highly DNA-reactive agent. Structurally related chemicals, also exhibit mutagenic and carcinogenic effects in laboratory animals.

Other Key Data

The structure and DNA reactivity of Substance 3 support potential carcinogenicity. Both properties are highly correlated with carcinogenicity. Numerous positive mutagenicity tests *in vitro* and *in vivo* add to this support and are reinforced by observation of similar genetic damage in exposed workers.

Substance 3 is experimentally observed to be readily absorbed by all routes and rapidly distributed through the body.

MODE OF ACTION

All of the available data in both humans and animals, strongly indicate a mutagenic mode of action, with a particular human target in lung tissue. A mechanistic linkage is found for rodents

and humans by observations of a similar profile of mutations in the p53 gene from the lung tumor tissue of the p53 transgenic mouse and exposed workers. This mutation spectra is consistent with the type of predominant DNA adducts induced by Substance 3. The tumor suppressor gene, p53, is a frequently mutated gene in human tumors, including lung. The consistent finding of mutagencity in experimental assays and human biomonitoring studies, the finding of p53 mutations in transgenic animal and human lung tumor tissue, all points to a mutagenic mode of action and supports assuming linearity of the dose-response relationship.

NARRATIVE #4

Substance #4

CAS# XXX

CANCER HAZARD SUMMARY

This chemical is *likely* to be carcinogenic to humans by *all routes* of exposure. Its carcinogenic potential is indicated by (a) tumor and toxicity studies on structural analogues, which demonstrate the ability of the chemical to produce thyroid follicular cell tumors in rats and hepatocellular tumors in mice following ingestion, and (b) metabolism and hormonal information on the chemical and its analogues, which contributes to a working mode of action and associates findings in animals with those in exposed humans. In comparison with other agents designated as likely carcinogens, the overall weight of evidence for this chemical places it at the *lower end* of the grouping. This is because there is a lack of tumor response data on this agent itself.

Biological information on the compound is contradictory in terms of how to quantitate potential cancer risks. The information on disruption on thyroid-pituitary status argues for using a margin of exposure evaluation. However, the chemical is an aromatic amine, a class of agents that are DNA-reactive and induce gene mutation and chromosome aberrations, which argues for low-dose linearity. Additionally, there is a lack of mode-of-action information on the mouse liver tumors produced by the structural analogues, also pointing toward a low-dose linear default approach. In recognition of these uncertainties, it is recommended to quantitate tumors using *both nonlinear* (to place a lower bound on the risks) *and linear* (to place an upper bound on the risks) *default approaches*. Given the absence of tumor response data on the chemical per se, it is recommended that tumor data on close analogues be used to possibly develop toxicity equivalent factors or relative potencies.

Overall, this chemical is an inferential case for potential human carcinogenicity. The uncertainties associated with this assessment include (1) the lack of carcinogenicity studies on the chemical, (2) the use of tumor data on structural analogues, (3) the lack of definitive information

on the relevance of thyroid-pituitary imbalance for human carcinogenicity, and (4) the different potential mechanisms that may influence tumor development and potential risks.

SUPPORTING INFORMATION

Human Data

Worker exposure has not been well characterized or quantified, but recent medical monitoring of workers exposed over a period of several years has uncovered alterations in thyroid-pituitary hormones (a decrease in T3 and T4 and an increase in TSH) and symptoms of hypothyroidism. A urinary metabolite of the chemical has been monitored in workers, with changes in thyroid and pituitary hormones noted, and the changes were similar to those seen in an animal study.

Animal Data

The concentration of the urinary metabolite in rats receiving the chemical for 28 days was within twofold of that in exposed workers, a finding associated with comparable changes in thyroid hormones and TSH levels. In addition, the dose of the chemical given to rats in this study was essentially the same as that of an analogue that had produced thyroid and pituitary tumors in rats. The human thyroid responds in the same way as the rodent thyroid following short-term, limited exposure. Although it is not well established that thyroid-pituitary imbalance leads to cancer in humans as it does in rodents, information in animals and in exposed humans suggests similar mechanisms of disrupting thyroid-pituitary function and the potential role of altered TSH levels in leading to thyroid carcinogenesis.

Structural Analogue Data

This chemical is an aromatic amine, a member of a class of chemicals that has regularly produced carcinogenic effects in rodents and gene and structural chromosome aberrations in short-term tests. Some aromatic amines have produced cancer in humans.

Close structural analogues produce thyroid follicular cell tumors in rats and hepatocellular tumors in mice following ingestion. The thyroid tumors are associated with known perturbations in thyroid-pituitary functioning. These compounds inhibit the use of iodide by the thyroid gland, apparently due to inhibition of the enzyme that synthesizes the thyroid hormones (T3, T4). Accordingly, blood levels of thyroid hormones decrease, which induces the pituitary gland to produce more TSH, a hormone that stimulates the thyroid to produce more of its hormones. The thyroid gland becomes larger because of increases in the size of individual cells and their

proliferation, and upon chronic administration of the chemical, tumors develop. Thus, thyroid tumor development is significantly influenced by disruption in the thyroid-pituitary axis.

Other Key Data

The chemical can be absorbed by the oral, inhalation, and dermal routes of exposure.

MODE OF ACTION

Data on the chemical and on structural analogues indicate the potential association of carcinogenesis with perturbation of thyroid-pituitary homeostasis. Structural analogues are genotoxic, thus raising the possibility of different mechanisms by which this chemical may influence tumor development.

NARRATIVE #5

Substance #5

CAS# XXX

CANCER HAZARD SUMMARY

Substance 5 is *likely* to be a human carcinogen by *all routes* of exposure. Findings are based on very extensive and significant experimental findings that include (a) tumors at multiple sites in both sexes of two rodent species via three routes of administration relevant to human exposure; (b) close structural analogues that produce a spectrum of tumors like those from Substance 5; (c) significant evidence for the production of reactive Substance 5 metabolites that readily bind to DNA and produce gene mutations in many systems, including cultured mammalian and human cells; and (d) two null studies and one positive epidemiologic study; in the positive study, there may have been exposure to Substance 5. These findings support a decision that Substance 5 might produce cancer in exposed humans. In comparison to other agents considered likely human carcinogens, the overall weight of evidence for Substance 5 puts it near the top of the grouping. Given the agent's mutagenicity, which can influence the carcinogenic process, a linear dose-response extrapolation is recommended.

Uncertainties include the lack of adequate information on the mutagenicity of Substance 5 in mammals or humans in vivo, although such effects would be expected.

SUPPORTING INFORMATION

Human Data

The information on the carcinogenicity of Substance 5 from human studies is inadequate. Two studies of production workers have not shown significant increases in cancer from exposure to Substance 5 and other chemicals. An increase in lymphatic cancer was reported in a mortality study of grain elevator workers who may have been exposed to Substance 5 (and other chemicals).

Animal Data

Substance 5 produced tumors in four chronic rodents studies. Tumor increases were noted in males and females of rats and mice following oral dermal and inhalation exposure (rat--oral and two inhalation, mouse--oral and dermal). It produces tumors both at the site of application (e.g., skin with dermal exposure) and at sites distal to the portal of entry into the body (e.g., mammary gland) following exposure from each route. Tumors at the same site were noted in both sexes of a species (blood vessel), both species (forestomach) and via different routes of administration (lung). Some tumors developed after very short latency, metastasized extensively, and produced death, an uncommon findings in rodents. The rodent studies were well designed and conducted except for the oral studies, in which the doses employed caused excessive toxicity and mortality. However, given the other rodent findings, lower doses would also be anticipated to be carcinogenic.

Structural Analogue Data

Several chemicals structurally related to Substance 5 are also carcinogenic in rodents. Among four that are closest in structure, tumors like those seen for Substance 5 were often noted (e.g., forestomach, mammary, lung), which helps to confirm the findings for Substance 5 itself. In sum, all of the tumor findings help to establish animal carcinogenicity and support potential human carcinogenicity for Substance 5.

Other Key Data

Substance 5 itself is not reactive, but from its structure it was expected to be metabolized to reactive forms. Extensive metabolism studies have confirmed this presumption and have demonstrated metabolites that bind to DNA and cause breaks in the DNA chain. These lesions are readily converted to gene mutations in bacteria, fungi, higher plants, insects, and mammalian and human cells in culture. There are only a limited number of reports on the induction of

chromosome aberrations in mammals and humans; thus far they are negative.

MODE OF ACTION

Human carcinogens often produce cancer in multiple sites of multiple animal species and both sexes and are mutagenic in multiple test systems. Substance 5 satisfies these findings. It produces cancer in males and females of rats and mice. It produces gene mutations in cells across all life forms--plants, bacteria, and animals--including mammals and humans. Given the mutagenicity of Substance 5 exposure and the multiplicity and short latency of Substance 5 tumor induction, it is reasonable to use a linear approach for cancer dose-response extrapolation.

APPENDIX B. RESPONSES TO THE NATIONAL ACADEMY OF SCIENCES NATIONAL RESEARCH COUNCIL REPORT *SCIENCE AND JUDGMENT IN RISK ASSESSMENT* (NRC, 1994)

Recommendations of the National Academy of Sciences National Research Council

In 1994, the National Academy of Sciences published a report, *Science and Judgment in Risk Assessment*. The report was written by a Committee on Risk Assessment of Hazardous Air Pollutants formed under the Academy's Board on Environmental Studies and Toxicology, Commission on Life Sciences, National Research Council. The report was called for under Section 112(o)(1)(A,B) of the Clean Air Act Amendments of 1990, which provided for the EPA to arrange for the Academy to review:

- risk assessment methodology used by EPA to determine the carcinogenic risk associated with exposure to hazardous air pollutants from source categories and subcategories subject to the requirements of this section, and
- improvements in such methodology.

Under Section 112(o)(2)(A,B), the Academy was to consider the following in its review:

- the techniques used for estimating and describing the carcinogenic potency to humans of hazardous air pollutants, and
- the techniques used for estimating exposure to hazardous air pollutants (for hypothetical and actual maximally exposed individuals as well as other exposed individuals).

To the extent practicable, the Academy was also to review methods of assessing adverse human health effects other than cancer for which safe thresholds of exposure may not exist [Section 112(o)(3)]. The Congress further provided that the EPA Administrator should consider, but need not adopt, the recommendations in the report and the views of the EPA Science Advisory Board with respect to the report. Prior to the promulgation of any standards under Section 112(f), the Administrator is to publish revised guidelines for carcinogenic risk assessment or a detailed explanation of the reasons that any recommendations contained in the report will not be implemented [Section 112(o)(6)].

The following discussion addresses the recommendations of the 1994 report that are pertinent to the EPA cancer risk assessment guidelines. Guidelines for assessment of exposure, of mixtures, and of other health effects are separate EPA publications. Many of the recommendations were related to practices specific to the exposure assessment of hazardous air

pollutants, which are not covered in cancer assessment guidelines. Recommendations about these other guidelines or practices are not addressed here.

Hazard Classification

The 1994 report contains the following recommendation about classifying cancer hazard:

- The EPA should develop a two-part scheme for classifying evidence on carcinogenicity that would incorporate both a simple classification and a narrative evaluation. At a minimum, both parts should include the strength (quality) of the evidence, the relevance of the animal model and results to humans, and the relevance of the experimental exposures (route, dose, timing, and duration) to those likely to be encountered by humans.

The report also presented a possible matrix of 24 boxes that would array weights of evidence against low, medium, or high relevance, resulting in 24 codes for expressing the weight and relevance.

These guidelines adopt five standard hazard descriptors and a narrative for presentation of the weight-of-evidence findings. The descriptors are used within the narrative. There is no matrix of alphanumerical weight-of-evidence boxes.

The issue of an animal model that is not relevant to humans has been dealt with by not including an irrelevant response in the weighing of evidence, rather than by creating a weight of evidence and then appending a discounting factor as the NRC scheme would do. The issue of relevance is more complex than the NRC matrix makes apparent. Often the question of relevance of the animal model applies to a single tumor response, but one encounters situations in which there are more tumor responses in animals than the questioned one. Dealing with this complexity is more straightforward if it is done during the weighing of evidence rather than after as in the NRC scheme. Moreover, the same experimental data are involved in deciding on the weight of evidence and the relevance of a response. It would be awkward to go over the same data twice.

In recommending that the relevance of circumstances of human exposure be taken into account, the NRC appears to assume that all of the actual conditions of human exposure will be known when the classification is done. This is not the case. More often than not, the hazard assessment is applied to risks associated with exposure to different media or environments at different times. In some cases, there is no priority to obtaining exposure data until the hazard assessment has been done. The approach of these guidelines is to characterize hazards as to whether their expression is intrinsically limited by route of exposure or by reaching a particular dose range based strictly on toxicological and other biological features of the agent. Both the use

of descriptors and the narrative specifically capture this information. Other aspects of appropriate application of the hazard and dose-response assessment to particular human exposure scenarios are dealt with in the characterization of the dose-response assessment, e.g., the applicability of the dose-response assessment to scenarios with differing frequencies and durations.

The NRC scheme apparently intended that the evidence would be weighed, then given a low, medium, or high code for some combination of relevance of the animal response, route of exposure, timing, duration, or frequency. The 24 codes contain none of this specific information, and, in fact, do not communicate what the conclusion is about. To make the codes communicate the information apparently intended would require some multiple of the 24 in the NRC scheme. As the number of codes increases, their utility for communication decreases.

Another reason for declining to use codes is that they tend to become outdated as research reveals new information that was not contemplated when they were adopted. This has been the case with the classification system under the 1986 EPA guidelines.

Even though these guidelines do not adopt a matrix of codes, their method of using descriptors and narratives captures the information the NRC recommended as the most important, and in the EPA's view, in a more transparent manner.

Dose-Response

The 1994 report contains the following recommendations about dose-response issues:

- EPA should continue to explore, and when scientifically appropriate, incorporate toxicokinetic models of the link between exposure and biologically effective dose (i.e., dose reaching the target tissue).
- Despite the advantages of developing consistent risk assessments between agencies by using common assumptions (e.g., replacing surface area with body weight to the 0.75 power), EPA should indicate other methods, if any, that would be more accurate.
- EPA should continue to use the linearized multistage model as a default option but should develop criteria for determining when information is sufficient to use an alternative extrapolation model.
- EPA should continue to use as one of its risk characterization metrics upper-bound potency estimates of the probability of developing cancer due to lifetime exposure. Whenever possible, this metric should be supplemented with other descriptions of cancer potency that might more adequately reflect the uncertainty associated with the estimates.

- EPA should adopt a default assumption for differences in susceptibility among humans in estimating individual risks.
- In the analysis of animal bioassay data on the occurrence of multiple tumor types, the cancer potencies should be estimated for each relevant tumor type that is related to exposure, and the individual potencies should be summed for those tumors.

Toxicokinetic models are encouraged in these guidelines, with discussion of appropriate considerations for their use. When there are questions as to whether such a model is more accurate in a particular case than the default method for estimating the human equivalent dose, both alternatives may be used. It should be noted that the default method for inhalation exposure is a toxicokinetic model.

The rationale for adopting the oral scaling factor of body weight to the 0.75 power has been discussed above in the explanation of major defaults. The empirical basis is further explored in U.S. EPA (1992b). The more accurate approach is to use a toxicokinetic model when data become available, or to modify the default when data are available, as encouraged under these guidelines. As the U.S. EPA (1992b) discussion explores in depth, data on the differences among animals in response to toxic agents are basically consistent with using a power of 1.0, 0.75, or 0.66. The Federal agencies chose the power of 0.75 for the scientific reasons given in the previous discussion of major defaults; these were not addressed specifically in the NRC report. It was also considered appropriate, as a matter of policy, for the agencies to agree on one factor. Again, the default for inhalation exposure is a model that is constructed to become better as more agent-specific data become available.

EPA proposes not to use a computer model such as the linearized multistage model as a default for extrapolation below the observed range. The reason is that the basis for default extrapolation is a theoretical projection of the likely shape of the curve, considering mode of action. For this purpose, a computer model looks more sophisticated than a straight-line extrapolation, but is not. The extrapolation will be by straight line as explained in the explanation of major defaults. This was also recommended by workshop reviewers of a previous draft of these guidelines (U.S. EPA, 1994b). In addition, a margin-of-exposure analysis is proposed in cases in which the curve is thought to be nonlinear, based on mode of action. In both cases, the observed range of data will be modeled by curve fitting in the absence of supporting data for a biologically based or case-specific model.

The result of using straight-line extrapolation is thought to be an upper bound on low-dose potency to the human population in most cases, but as discussed in the major defaults section, it may not always be. Exploration and discussion of uncertainty of parameters in curve-

fitting a model of the observed data or in using a biologically based or case-specific model is called for in the dose-response assessment and characterization sections of these guidelines.

The issue of a default assumption for human differences in susceptibility has been addressed under the major defaults discussion in Section 1.3 with respect to margin-of-exposure analysis. EPA has considered but decided not to adopt a quantitative default factor for human differences in susceptibility when a linear extrapolation is used. In general, EPA believes that linear extrapolation is sufficiently conservative to protect public health. Linear approaches (both LMS and straight-line extrapolation) from animal data are consistent with linear extrapolation on the same agents from human data (Goodman and Wilson, 1991; Hoel and Portier, 1994). If actual data on human variability in sensitivity are available they will, of course, be used.

In analyzing animal bioassay data on the occurrence of multiple tumor types, these guidelines outline a number of biological and other factors to consider. The objective is to use these factors to select response data (including nontumor data as appropriate) that best represent the biology observed. As stated in Section 3 of the guidelines, appropriate options include use of a single data set, combining data from different experiments, showing a range of results from more than one data set, showing results from analysis of more than one tumor response based on differing modes of action, representing total response in a single experiment by combining animals with tumors, or a combination of these options. The approach judged to best represent the data is presented with the rationale for the judgment, including the biological and statistical considerations involved. EPA has considered the approach of summing tumor incidences and decided not to adopt it. While multiple tumors may be independent, in the sense of not arising from metastases of a single malignancy, it is not clear that they can be assumed to represent different effects of the agent on cancer processes. In this connection, it is not clear that summing incidences provides a better representation of the underlying mode(s) of action of the agent than combining animals with tumors or using another of the several options noted above. Summing incidences would result in a higher risk estimate, a step that appears unnecessary without more reason.

Risk Characterization

- When EPA reports estimates of risk to decisionmakers and the public, it should present not only point estimates of risk, but also the sources and magnitudes of uncertainty associated with these estimates.
- Risk managers should be given characterizations of risk that are both qualitative and quantitative, i.e., both descriptive and mathematical.

- EPA should consider in its risk assessments the limits of scientific knowledge, the remaining uncertainties, and the desire to identify errors of either overestimation or underestimation.

In part as a response to these recommendations, the Administrator of EPA issued guidelines for risk characterization and required implementation plans from all programs in EPA (U.S. EPA, 1995). The Administrator's guidance is followed in these cancer guidelines. The assessments of hazard, dose-response, and exposure will all have accompanying technical characterizations covering issues of strengths and limitations of data and current scientific understanding, identification of defaults utilized in the face of gaps in the former, discussions of controversial issues, and discussions of uncertainties in both their qualitative and, as practicable, their quantitative aspects.

APPENDIX C. CASE STUDY EXAMPLES FOR HAZARD EVALUATION

This section provides examples of substances that fit the descriptors above. These examples are based on available information about real substances and are selected to illustrate the principles for weight-of-evidence evaluation and the application of the classification scheme.

These case studies show the interplay of differing lines of evidence in making a conclusion. Some particularly illustrate the role that "other key data" can play in conclusions.

Example 1: "Carcinogenic to Humans"--Route-Dependent/Linear Extrapolation

Human Data

Substance 1 is an aluminosilicate mineral that exists in nature with a fibrous habit. Several descriptive epidemiologic studies have demonstrated very high mortality from malignant mesothelioma, mainly of the pleura, in three Turkish villages where there was a contamination of this mineral and where exposure had occurred from birth. Both sexes were equally affected and at an unusually young age.

Animal Data

Substance 1 has been studied in a single long-term inhalation study in rats at one exposure concentration that showed an extremely high incidence of pleural mesothelioma (98% in treated animals versus 0% in concurrent controls). This is a rare malignant tumor in the rat and the onset of tumors occurred at a very early age (as early as 1 year). Several studies involving injection into the body cavities of rats or mice (i.e., pleural or peritoneal cavities) also produced high incidences of pleural or peritoneal mesotheliomas. No information is available on the carcinogenic potential of substance 1 in laboratory animals via oral and dermal exposures.

Other Key Data

Information on the physical and chemical properties of substance 1 indicates that it is highly respirable to humans and laboratory rodents. It is highly insoluble and is not likely to be readily degraded in biological fluid.

No information is available on the deposition, translocation, retention, lung clearance, and excretion of the substance after inhalation exposure or ingestion. Lung burden studies have shown the presence of elevated levels of the substance in lung tissue samples of human cases of pleural mesotheliomas from contaminated villages compared with control villages.

No data are available on genetic or related effects in humans. The substance has been shown to induce unscheduled DNA synthesis in human cells in vitro and transformation and unscheduled DNA synthesis in mouse cells.

The mechanisms by which this substance causes cancer in humans and animals are not understood, but appear to be related to its unique physical, chemical, and surface properties. Its fiber morphology is similar to a known group of naturally occurring silicate minerals that have been known to cause respiratory cancers in humans(including pleural mesothelioma) from inhalation exposure and genetic changes.

Evaluation

Human evidence is judged to establish a causal link between exposure to substance 1 and human cancer. Even though the human evidence does not satisfy all criteria for causality, this judgment is based on a number of unusual observations: large magnitude of the association, specificity of the association, demonstration of environmental exposure, biological plausibility, and coherence based on the entire body of knowledge of the etiology of mesothelioma.

Animal evidence demonstrates a causal relationship between exposure and cancer in laboratory animals. Although available data are not optimal in terms of design (e.g., the use of single dose, one sex only), the judgment is based on the unusual findings from the only inhalation experiment in rats (i.e., induction of an uncommon tumor, an extremely high incidence of malignant neoplasms, and onset of tumors at an early age). Additional evidence is provided by consistent results from several injection studies showing an induction of the same tumors by different modes of administration in more than one species.

Other key data, while limited, support the human and animal evidence of carcinogenicity. It can be inferred from human and animal data that this substance is readily deposited in the respiratory airways and deep lung and is retained for extended periods of time after first exposure. Information on related fibrous substances indicates that the modes of action are likely mediated by the physical and chemical characteristics of the substance (e.g., fiber shape, high aspect ratio, a high degree of insolubility in lung tissues).

Insufficient data are available to evaluate the human carcinogenic potential of substance 1 by oral exposure. Even though there is no information on its carcinogenic potential via dermal uptake, it is not expected to pose a carcinogenic hazard to humans by that route because it is very insoluble and is not likely to penetrate the skin.

Conclusion

It is concluded that substance 1 is *carcinogenic to humans by inhalation exposure*. The weight of evidence of human carcinogenicity is based on (a) exceptionally increased incidence of malignant mesothelioma in epidemiologic studies of environmentally exposed human populations; (b) significantly increased incidence of malignant mesothelioma in a single inhalation study in rats and in several injection studies in rats and mice; and (c) supporting information on related fibrous substances that are known to cause cancer via inhalation and genetic damage in exposed mammalian and human mesothelial cells. The human carcinogenic potential of substance 1 via oral exposure cannot be determined on the basis of insufficient data. It is not likely to pose a carcinogenic hazard to humans via dermal uptake because it is not anticipated to penetrate the skin.

The mode of action of this substance is not understood. In addition to this uncertainty, dose-response information is lacking for both human and animal data. Epidemiologic studies contain observations of significant excess cancer risks at relatively low levels of environmental exposure. The use of *linear* extrapolation in a dose-response relationship assessment is appropriate as a default since mode-of-action data are not available.

Example 2: "Carcinogenic to Humans"-- Any Exposure Conditions/Linear Extrapolation

Human Data

Substance 2 is an alkylating agent that is used extensively as a chemical intermediate in organic synthesis, particularly in the synthesis of plastics and resins. Several cohort studies of workers using substance 2 have been conducted. Four studies of chemical workers exposed to substance 2 (as well as other agents) found an increased mortality rate from lung cancer. The excess was primarily found in small subgroups with high-level exposure. Although smoking was a confounding factor, the predominant lung tumor found was small-cell carcinoma, which is distinct from the squamous cell carcinomas usually found in smokers. Although the type of lung cancer was consistent among the four studies, the dose-response, latency period, and average age of appearance was not consistent. Furthermore, there are confounding exposures to other chemicals. No increase in mortality rate was observed in two studies, one of which had exposures higher than the studies reporting an increased incidence of lung cancer.

Animal Data

A multisite tumor response in rats and mice of both sexes is found in 2-year rodent bioassay studies when substance 2 is administered by various routes. In particular, the induction of lung tumors is consistently found across different studies, species, and routes of administration. For example, when administered by inhalation, substance 2 induced a dose-related increase in the incidences of lung tumors in female and male mice (B6C3F1); and squamous cell carcinomas of the lung and nasal tumors in male rats (F344). When administered by subcutaneous injection, substance 2 induced a statistically significant response for pulmonary tumors and local fibrosarcomas in mice of both sexes. An oral gavage 2-year study resulted in an elevated incidence of lung tumors in male rats and both sexes of mice, forestomach tumors in both sexes of rats and mice, liver tumors in both sexes of rats, and urinary bladder tumors in both sexes of mice. Substance 2 produced lung and forestomach tumors in the p53 mouse cancer transgenic assay when administered via gavage. It is an initiator of skin tumors in mice.

Other Key Data

Substance 2 is a liquid but can exist as a vapor at room temperature given its high vapor pressure. It is readily absorbed dermally. Studies in rats indicate that, once absorbed, substance 2 is uniformly distributed throughout the body. It is metabolized by hydrolysis and by conjugation with glutathione. The ability to form glutathione conjugate varies across animal species, with the rat being most active, followed by mice.

Substance 2 induces cell transformation in the Syrian Hamster Embryo assay. It is a direct-acting alkylating agent and is consistently mutagenic when tested in a variety of nonmammalian and mammalian assays, including in vivo rodent tests. It has been shown to form DNA adducts and to produce predominantly GC to AT transitions. Substance 2 produces similar genetic lesions in rodents and humans. It was found to cause dose-related increases, HPRT mutations, and chromosome aberrations in peripheral blood lymphocytes of exposed workers. A similar p53 mutation spectra has been found in lung tumor tissue from the p53 transgenic mouse and human cancer biopsies. In humans, a large number of the cases had a mutation in p53, with a predominance of GC to AT transitions. The mutation spectra of substance-2-associated lung tumors differed from patterns reported for sporadic and smoking-related tumors.

SAR analysis indicates that substance 2 is a highly DNA-reactive agent. Structurally related chemicals also exhibit mutagenic and carcinogenic effects in laboratory animals.

Evaluation

Available epidemiologic studies, taken together, suggest that a causal association between exposure to substance 2 and elevated risk of cancer is plausible. This judgment is based on small but consistent excesses of lung tumors that are distinct from smoking-related lung cancer in the studies of highly exposed workers. The evidence is close and indicates that causal interpretation is credible, but not conclusively demonstrated because of certain inconsistencies in the available studies, possible bias, and confounding factors that could not be adequately excluded.

Extensive evidence indicates that substance 2 is carcinogenic to laboratory animals in multiple species and at multiple tissue sites with multiple routes of exposure. There is an induction of malignant tumors to an unusual degree with regard to incidence. In particular, there is a consistent dose-related induction of lung tumors across different species and routes of administration in well-designed and conducted studies. This tumor response is similar to that reported in exposed humans.

The potential human carcinogenicity of substance 2 is reinforced by observations of similar genetic damage (DNA adducts, HPRT mutations, chromosomal aberrations) in experimental tests and exposed workers. The genetic effects induced in experimental animals are dose related and observed at exposures lower than those that produce lung tumors in rodent bioassays. A mechanistic linkage is found for rodents and humans by observations of a similar profile of mutations in the p53 gene from the lung tumor tissue of the p53 transgenic mouse and exposed workers. This mutation spectra is consistent with the type of predominant DNA adducts induced by substance 2.

Substance 2 belongs to a well-defined, structurally related class of substances whose members are carcinogenic in rodents and are likely to be human carcinogens.

Conclusion

It is concluded that substance 2 is ***"carcinogenic to humans"*** by *all routes of exposure*. The weight of evidence of human carcinogenicity is based on (a) consistent evidence of carcinogenicity in rats and mice by oral and inhalation exposure; (b) epidemiologic evidence suggestive of a causal association between exposure and elevated risk of lung cancer, which is the tumor type consistently induced in different test species and with different routes of administration; (c) evidence of genetic damage in blood lymphocytes of exposed workers; (d) mutagenic effects in numerous in vivo and in vitro test systems, which are similar to those found in humans; (e) similar profile of p53 mutations in rodent and human lung tumor tissue; (f) membership in a class of DNA-reactive compounds that have been shown to cause carcinogenic

and mutagenic effects in animals; and (g) ability to be absorbed by all routes of exposure, followed by rapid distribution throughout the body.

The evidence is compelling that the mutagenic properties of substance 2 in experimental animals and humans are an important influence on the carcinogenic process. Thus, substance 2 acts through a mode of action that is operative in humans and would therefore reasonably be anticipated to cause cancer in humans. A linear extrapolation should be assumed in dose-response assessment.

Example 3: "Likely Human Carcinogen"--Any Exposure Conditions/Linear Extrapolation

Human Data

Substance 3 is a brominated alkane. Three studies have investigated the cancer mortality of workers exposed to this substance. No statistically significant increase in cancer at any site was found in a study of production workers exposed to substance 3 and several other chemicals. Elevated cancer mortality was reported in a much smaller study of production workers. An excess of lymphoma was reported in grain workers who may have had exposure to substance 3 and other chemical compounds. These studies are considered inadequate due to their small cohort size; lack of or poorly characterized exposure concentrations; or concurrent exposure of the cohort to other potential or known carcinogens.

Animal Data

The potential carcinogenicity of substance 3 has been extensively studied in an oral gavage study in rats and mice of both sexes, two inhalation studies of rats of different strains of both sexes, an inhalation study in mice of both sexes, and a skin painting study in female mice.

In the oral study, increased incidences of squamous-cell carcinoma of the forestomach were found in rats and mice of both sexes. Additionally, there were increased incidences of liver carcinomas in female rats, hemangiosarcomas in male rats, and alveolar/bronchiolar adenoma of the lung of male and female mice. Excessive toxicity and mortality were observed in the rat study, especially in the high-dose groups, which resulted in early termination of the study, and similar time-weighted average doses for the high- and low-treatment groups.

In the first inhalation study in rats and mice, increased incidences of carcinomas and adenocarcinomas of the nasal cavity and hemangiosarcoma of the spleen were found in exposed animals of each species of both sexes. Treated female rats also showed increased incidences of alveolar/bronchiolar carcinoma of the lung and mammary gland fibroadenomas. Treated male rats showed an increased incidence of peritoneal mesothelioma. In the second inhalation study in rats

(single exposure only), significantly increased incidences of hemangiosarcoma of the spleen and adrenal gland tumors were seen in exposed animals of both sexes. Additionally, increased incidences of subcutaneous mesenchymal tumors and mammary gland tumors were induced in exposed male and female rats, respectively.

Lifetime dermal application of substance 3 to female mice resulted in significantly increased incidences of skin papillomas and lung tumors.

Several chemicals structurally related to substance 3 are also carcinogenic in rodents. The spectrum of tumor responses induced by related substances was similar to those seen with substance 3 (e.g., forestomach, mammary gland, and lung tumors).

Other Key Data

Substance 3 exists as a liquid at room temperature and is readily absorbed by ingestion, inhalation, and dermal contact. It is widely distributed in the body and is eliminated in the urine mainly as metabolites (e.g., glutathione conjugate).

Substance 3 is not itself DNA-reactive, but is biotransformed to reactive metabolites, as inferred by findings of its covalent binding to DNA and induction of DNA strand breaks, both in vivo and in vitro. Substance 3 has been shown to induce sister chromatid exchanges, mutations, and unscheduled DNA synthesis in human and rodent cells in vitro. Reverse and forward mutations have been consistently produced in bacterial assays and in vitro assays using eukaryotic cells. Substance 3, however, did not induce dominant lethal mutations in mice or rats, or chromosomal aberrations or micronuclei in bone marrow cells of mice treated in vivo.

Evaluation

Available epidemiologic data are considered inadequate for an evaluation of a causal association of exposure to the substance and excess of cancer mortality due to major study limitations.

There is extensive evidence that substance 3 is carcinogenic in laboratory animals. Increased incidences of tumors at multiple sites have been observed in multiple studies in two species of both sexes with different routes of exposure. It induces tumors both at the site of entry (e.g., nasal tumors via inhalation, forestomach tumors by ingestion, skin tumors with dermal exposure) and at distal sites (e.g., mammary gland tumors). Additionally, it induced tumors at the same sites in both species and sexes via different routes of exposure (e.g., lung tumors). With the exception of the oral study in which the employed doses caused excessive toxicity and mortality, the other studies are considered adequately designed and well conducted. Overall, given the

magnitude and extent of animal carcinogenic responses to substance 3, coupled with similar responses to structurally related substances, these animal findings are judged to be highly relevant and predictive of human responses.

Other key data, while not very extensive, are judged to be supportive of carcinogenic potential. Substance 3 has consistently been shown to be mutagenic in mammalian cells, including human cells, and in nonmammalian cells; thus, mutation is likely a mode of action for its carcinogenic activity. However, the possible involvement of other modes of action has not been fully investigated. Furthermore, induction of genetic changes from in vivo exposure to substance 3 has not been demonstrated.

Conclusion

Substance 3 is *likely to be carcinogenic to humans*. In comparison with other agents designated as likely human carcinogens, the overall weight of evidence for substance 3 puts it at the *high end* of the grouping.

The weight of evidence of human carcinogenicity is based on animal evidence and other key evidence. Human data are inadequate for an evaluation of human carcinogenicity. The overall weight of evidence is based on (a) extensive animal evidence showing induction of increases of tumors at multiple sites in both sexes of two rodent species via three routes of administration relevant to human exposure; (b) tumor data of structural analogues exhibiting similar patterns of tumors in treated rodents; (c) in vitro evidence for mutagenic effects in mammalian cells and nonmammalian systems; and (d) its ability to be absorbed by all routes of exposure followed by rapid distribution throughout the body.

Some uncertainties are associated with the mechanisms of carcinogenicity of substance 3. Although there is considerable evidence indicating that mutagenic events could account for carcinogenic effects, there is still a lack of adequate information on the mutagenicity of substance 3 in vivo in animals or humans. Moreover, alternative modes of action have not been explored. Nonetheless, available data indicate a likely mutagenic mode of action. Linear extrapolation should be assumed in dose-response assessment.

Example 4: "Likely Human Carcinogen"--All Routes/Linear and Nonlinear Extrapolation

Human Data

Substance 4 is a chlorinated alkene solvent. Several cohort studies of dry cleaning and laundry workers exposed to substance 4 and other solvents reported significant excesses of mortality due to cancers of the lung, cervix, esophagus, kidney, bladder, lymphatic and

hematopoietic system, colon, or skin. No significant cancer risks were observed in a subcohort of one of these investigations of dry cleaning workers exposed mainly to substance 4. Possible confounding factors such as smoking, alcohol consumption, or low socioeconomic status were not considered in the analyses of these studies.

A large case-control study of bladder cancer did not show any clear association with dry cleaning. Several case-control studies of liver cancer identified an increased risk of liver cancer with occupational exposure to organic solvents. The specific solvents to which workers were exposed and exposure levels were not identified.

Animal Data

The potential carcinogenicity of substance 4 has been investigated in two long-term studies in rats and mice of both sexes by oral administration and inhalation.

Significant increases in hepatocellular carcinomas were induced in mice of both sexes treated with substance 4 by oral gavage. No increases in tumor incidence were observed in treated rats. Limitations in both experiments included control groups smaller than treated groups, numerous dose adjustments during the study, and early mortality due to treatment-related nephropathy.

In the inhalation study, there were significantly increased incidences of hepatocellular adenoma and carcinoma in exposed mice of both sexes. In rats of both sexes, there were marginally significant increased incidences of mononuclear cell leukemia (MCL) when compared with concurrent controls. The incidences of MCL in control animals, however, were higher than historical controls from the conducting laboratory. The tumor finding was also judged to be biologically significant because the time to onset of tumor was decreased and the disease was more severe in treated than in control animals. Low incidences of renal tubular cell adenomas or adenocarcinomas were also observed in exposed male rats. The tumor incidences were not statistically significant, but there was a significant trend.

Other Key Data

Substance 4 has been shown to be readily and rapidly absorbed by inhalation and ingestion in humans and laboratory animals. Absorption by dermal exposure is slow and limited. Once absorbed, substance 4 is primarily distributed to and accumulated in adipose tissue and the brain, kidney, and liver. A large percentage of substance 4 is eliminated unchanged in exhaled air, with urinary excretion of metabolites comprising a much smaller percentage. The absorption and distribution profiles of substance 4 are similar across species including humans.

Two major metabolites (trichloroacetic acid [TCA] and trichloroethanol), which are formed by a P-450-dependent mixed-function oxidase enzyme system, have been identified in all studied species, including humans. There is suggestive evidence for the formation of an epoxide intermediate based on the detection of two other metabolites (oxalic acid and trichloroacetyl amide). In addition to oxidative metabolism, substance 4 also undergoes conjugation with glutathione. Further metabolism by renal beta-lyases could lead to two minor active metabolites (trichlorovinyl thiol and dichlorothiokente).

Toxicokinetic studies have shown that the enzymes responsible for the metabolism of substance 4 can be saturated at high exposures. The glutathione pathway was found to be a minor pathway at low doses, but more prevalent following saturation of the cytochrome P-450 pathway. Comparative in vitro studies indicate that mice have a greater capacity to metabolize to TCA than rats and humans. Inhalation studies also indicate saturation of oxidative metabolism of substance 4, which occurs at higher dose levels in mice than in rats and humans. Based on these findings, it has been postulated that the species differences in the carcinogenicity of substance 4 between rats and mice may be related to the differences in the metabolism to TCA and glutathione conjugates.

Substance 4 is a member of the class of chlorinated organics that often cause liver and kidney toxicity and carcinogenesis in rodents. Like many chlorinated organics, substance 4 itself does not appear to be mutagenic. Substance 4 was generally negative in in vitro bacterial systems and in vivo mammalian systems. However, a minor metabolite formed in the kidney by the glutathione conjugation pathway has been found to be a strong mutagen.

The mechanisms of induced carcinogenic effects of substance 4 in rats and mice are not completely understood. It has been postulated that mouse liver carcinogenesis is related to liver peroxisomal proliferation and toxicity of the metabolite TCA. Information on whether or not TCA induces peroxisomal proliferation in humans is not definitive. The induced renal tumors in male rats may be related either to kidney toxicity or the activity of a mutagenic metabolite. The mechanisms of increases in MCL in rats are not known.

Evaluation

Available epidemiologic studies, taken together, provide suggestive evidence of a possible causal association between exposure to substance 4 and cancer incidence in the laundry and dry cleaning industries. This is based on consistent findings of elevated cancer risks in several studies of different populations of dry cleaning and laundry workers. However, each individual study is compromised by a number of study deficiencies including small numbers of cancers, confounding

exposure to other solvents, and poor exposure characterization. Others may interpret these findings collectively as inconclusive.

There is considerable evidence that substance 4 is carcinogenic to laboratory animals. It induces tumors in mice of both sexes by oral and inhalation exposure and in rats of both sexes via inhalation. However, owing to incomplete understanding of the mode of action, the predictivity of animal responses to humans is uncertain.

Animal data of structurally related compounds showing common target organs of toxicity and carcinogenic effects (but lack of mutagenic effects) provide additional support for the carcinogenicity of substance 4. Comparative toxicokinetic and metabolism information indicates that the mouse may be more susceptible to liver carcinogenesis than rats and humans. This may indicate differences of the degree and extent of carcinogenic responses, but does not detract from the qualitative weight of evidence of human carcinogenicity. The toxicokinetic information also indicates that oral and inhalation are the major routes of human exposure.

Conclusion

Substance 4 is *likely to be carcinogenic to humans by all routes of exposure*. The weight of evidence of human carcinogenicity is based on: (a) demonstrated evidence of carcinogenicity in two rodent species of both sexes via two relevant routes of human exposure; (b) the substance's similarity in structure to other chlorinated organics that are known to cause liver and kidney toxicity and carcinogenesis in rodents; (c) suggestive evidence of a possible association between exposure to the substance in the laundry and dry cleaning industries and increased cancer incidence; and (d) human and animal data indicating that the substance is absorbed by all routes of exposure.

There is considerable scientific uncertainty about the human significance of certain rodent tumors associated with substance 4 and related compounds. In this case, the human relevance of the animal evidence of carcinogenicity relies on the default assumption.

Overall, there is not enough evidence to give high confidence in a conclusion about any single mode of action; it appears that more than one is plausible in different rodent tissues. Nevertheless, the lack of mutagenicity of substance 4 and its general growth-promoting effect on high background tumors, as well as its toxicity toward mouse liver and rat kidney tissue, support the view that the predominant mode is growth-promoting rather than mutagenic. A mutagenic contribution to carcinogenicity due to a metabolite cannot be ruled out. The dose-response assessment should, therefore, adopt both default approaches, nonlinear and linear extrapolations.

The latter approach is very conservative since it likely overestimates risk at low doses in this case, and is primarily useful for screening analyses.

Example 5: "Likely/Not Likely Human Carcinogen"--Range of Dose Limited, Margin-of-Exposure Extrapolation

Human Data

Substance 5 is a metal-conjugated phosphonate. No human tumor or toxicity data exist on this chemical.

Animal Data

Substance 5 caused a statistically significant increase in the incidence of urinary bladder tumors in male, but not female, rats at 30,000 ppm (3%) in the diet in a long-term study. Some of these animals had accompanying urinary tract stones and toxicity. No bladder tumors or adverse urinary tract effects were seen in two lower dose groups (2,000 and 8,000 ppm) in the same study. A chronic dietary study in mice at doses comparable to those in the rat study showed no tumor response or urinary tract effects. A 2-year study in dogs at doses up to 40,000 ppm showed no adverse urinary tract effects.

Other Key Data

Subchronic dosing of rats confirmed that there was profound development of stones in the male bladder at doses comparable to those causing cancer in the chronic study, but not at lower doses. Sloughing of the epithelium of the urinary tract accompanied the stones.

There was a lack of mutagenicity relevant to carcinogenicity. In addition, there is nothing about the chemical structure of substance 5 to indicate DNA reactivity or carcinogenicity.

Substance 5 is composed of a metal, an ethanol, and a simple phosphorus-oxygen-containing component. The metal is not absorbed from the gut, whereas the other two components are absorbed. At high doses, ethanol is metabolized to carbon dioxide, which makes the urine more acidic; the phosphorus level in the blood and calcium in the urine are increased. Chronic testing of the phosphorus-oxygen-containing component alone in rats did not show any tumors or adverse effects on the urinary tract.

Because substance 5 is a metal complex, it is not likely to be readily absorbed from the skin.

Evaluation

Substance 5 produced cancer of the bladder and urinary tract toxicity in male, but not female rats and mice, and dogs failed to show the toxicity noted in male rats. The mode of action developed from the other key data to account for the toxicity and tumors in the male rats is the production of bladder stones. At high but not lower subchronic doses in the male rat, substance 5 leads to elevated blood phosphorus levels; the body responds by releasing excess calcium into the urine. The calcium and phosphorus combine in the urine and precipitate into multiple stones in the bladder. The stones are very irritating to the bladder; the bladder lining is eroded and cell proliferation occurs to compensate for the loss of the lining. Cell layers pile up, and finally, tumors develop. Stone formation does not involve the chemical per se but is secondary to the effects of its constituents on the blood and, ultimately, the urine. Bladder stones, regardless of their cause, commonly produce bladder tumors in rodents, especially the male rat.

Conclusion

Substance 5, a metal aliphatic phosphonate, is *likely to be carcinogenic to humans* only under high-exposure conditions following *oral and inhalation exposure* that lead to bladder stone formation, but is *not likely* to be carcinogenic under low-exposure conditions. It is *not likely to be a human carcinogen* via the *dermal* route, given that the compound is a metal conjugate that is readily ionized and its dermal absorption is not anticipated. The weight of evidence is based on (a) bladder tumors only in male rats; (b) the absence of tumors at any other site in rats or mice; (c) the formation of calcium-phosphorus-containing bladder stones in male rats at high, but not low, exposures that erode bladder epithelium and result in profound increases in cell proliferation and cancer; and (d) the absence of structural alerts or mutagenic activity.

There is a strong mode-of-action basis for the requirements of (a) high doses of substance 5, (b) which lead to excess calcium and increased acidity in the urine, (c) which result in the precipitation of stones, and (d) the necessity of stones for toxic effects and tumor hazard potential. Lower doses fail to perturb urinary constituents, lead to stones, produce toxicity, or give rise to tumors. Therefore, dose-response assessment should assume nonlinearity.

A major uncertainty is whether the profound effects of substance 5 may be unique to the rat. Even if substance 5 produced stones in humans, there is only limited evidence that humans with bladder stones develop cancer. Most often human bladder stones are either passed in the urine or lead to symptoms resulting in their removal. However, since one cannot totally dismiss the male rat findings, some hazard potential may exist in humans following intense exposures. Only fundamental research could illuminate this uncertainty.

Example 6: "Suggestive" Evidence

Human Data

Substance 6 is an unsaturated aldehyde. In a cohort study of workers in a chemical plant exposed to a mixture of chemicals with substance 6 as a minor component, a greater risk of cancer was reported than was expected. This study is considered inadequate because of multiple exposures, small cohort, and poor exposure characterization.

Animal Data

Substance 6 was tested for potential carcinogenicity in a drinking water study in rats, an inhalation study in hamsters, and a skin painting study in mice. No significant increases in tumors were observed in male rats treated with substance 6 at three dose levels in drinking water. However, a significant increase of adrenal cortical adenomas was found in the only treated female dose group administered a dose equivalent to the high dose of males. This study used a small number of animals (20 per dose group).

No significant finding was detected in the inhalation study in hamsters. This study is inadequate due to the use of too few animals, short duration of exposure, and inappropriate dose selection (use of a single exposure that was excessively toxic as reflected by high mortality).

No increase in tumors was induced in the skin painting study in mice. This study is of inadequate design for carcinogenicity evaluation because of several deficiencies: small number of animals, short duration of exposure, lack of reporting about the sex and age of animals, and purity of test material.

Substance 6 is structurally related to low-molecular-weight aldehydes that generally exhibit carcinogenic effects in the respiratory tracts of laboratory animals via inhalation exposure. Three skin painting studies in mice and two subcutaneous injection studies of rats and mice were conducted to evaluate the carcinogenic potential of a possible metabolite of substance 6 (identified in vitro). Increased incidences of either benign or combined benign and malignant skin tumors were found in the dermal studies. In the injection studies of rats and mice, increased incidences of local sarcomas or squamous cell carcinoma were found at the sites of injection. All of these studies are limited by the small number of test animals, the lack of characterization of test material, and the use of single doses.

Other Key Data

Substance 6 is a flammable liquid at room temperature. Limited information on its toxicokinetics indicates that it can be absorbed by all routes of exposure. It is eliminated in the

urine mainly as glutathione conjugates. Substance 6 is metabolized in vitro by rat liver and lung microsomal preparations to a dihydroxylated aldehyde.

No data were available on the genetic and related effects of substance 6 in humans. It did not induce dominant lethal mutations in mice. It induced sister chromatid exchanges in rodent cells in vitro. The mutagenicity of substance 6 is equivocal in bacteria. It did not induce DNA damage or mutations in fungi.

Evaluation

Available human data are judged *suggestive, but not sufficient* for an evaluation of any causal relationship between exposure to substance 6 and human cancer.

The carcinogenic potential of substance 6 has not been adequately studied in laboratory animals due to serious deficiencies in study design, especially the inhalation and dermal studies. There is suggestive evidence of carcinogenicity in the drinking water study in female rats. However, the significance of that study to a potential for human response is uncertain since the finding is limited to occurrence of benign tumors in one sex, and at the high dose only. Additional suggestion for animal carcinogenicity comes from observation that a possible metabolite is carcinogenic at the site of administration. This metabolite, however, has not been studied in vivo. Overall, the animal evidence is judged to be suggestive for human carcinogenicity.

Other key data, taken together, do not add significantly to the overall weight of evidence of carcinogenicity. SAR analysis indicates that substance 6 would be DNA-reactive. However, mutagenicity data are inconclusive. Limited in vivo data do not support a mutagenic effect. While there is some evidence of DNA damage in rodent cells in vitro, there is either equivocal or no evidence of mutagenicity in nonmammalian systems.

Conclusion

While there is a suggestion of animal carcinogenicity, the data are inadequate for a judgment about the human carcinogenicity potential of substance 6. Both human and animal data are judged inadequate for an evaluation. There is evidence suggestive of potential carcinogenicity on the basis of limited animal findings and SAR considerations. Data are not sufficient to judge whether there is a mode of carcinogenic action. Additional studies are needed for a full evaluation of the potential carcinogenicity of substance 6. Hence, dose-response assessment is not appropriate.

Example 7: "Not Likely to be a Human Carcinogen"--Appropriately Studied Chemical in Animals Without Tumor Effects

Human Data

Substance 7, a plant extract, has not been studied for its toxic or carcinogenic potential in humans.

Animal Data

Substance 7 has been studied in four chronic studies in three rodent species. In a feeding study in rats, males showed a nonsignificant increase in benign tumors of the parathyroid gland in the high-dose group, where the incidence in concurrent controls greatly exceeded the historical control range. Females demonstrated a significant increase in various subcutaneous tumors in the low-dose group, but findings were not confirmed in the high-dose group, and there was no dose-response relationship. These effects were considered as not adding to the evidence of carcinogenicity. No tumor increases were noted in a second adequate feeding study in male and female rats. In a mouse feeding study, no tumor increases were noted in dosed animals. There was some question as to the adequacy of the dosing; however, it was noted that in the mouse 90-day subchronic study that a dose of twice the high dose in the chronic study led to significant decrements in body weight. In a hamster study there were no significant increases in tumors at any site. No structural analogues of substance 7 have been tested for cancer.

Other Key Data

There are no structural alerts that would suggest that substance 7 is a DNA-reactive compound. It is negative for gene mutations in bacteria and yeast, but positive in cultured mouse cells. Tests for structural chromosome aberrations in cultured mammalian cells and in rats are negative; however, the animals were not tested at sufficiently high doses. Substance 7 binds to proteins of the cell division spindle; therefore, there is some likelihood for producing numerical chromosome aberrations, an endpoint that is sometimes noted in cancers. In sum, there is limited and conflicting information concerning the mutagenic potential of the agent.

The compound is absorbed via oral and inhalation exposure but only poorly via the skin.

Evaluation

The only indication of a carcinogenic effect comes from the finding of benign tumors in male rats in a single study. There is no confirmation of a carcinogenic potential from dosed

females in that study, in males and females in a second rat study, or from mouse and hamster studies.

There is no structural indication that substance 7 is DNA-reactive, there is inconsistent evidence of gene mutations, and chromosome aberration testing is negative. The agent binds to cell division spindle proteins and may have the capacity to induce numerical chromosome anomalies. Further information on gene mutations and in vivo structural and numerical chromosome aberrations may be warranted.

Conclusion

Substance 7 is *not likely to be carcinogenic to humans* via all relevant routes of exposure. This weight-of-evidence judgment is largely based on the absence of significant tumor increases in chronic rodent studies. Adequate cancer studies in rats, mice, and hamsters fail to show any carcinogenic effect; a second rat study showed an increase in benign tumors at a site in dosed males, but not females.

APPENDIX D. CASE STUDY EXAMPLES FOR MODE-OF-ACTION EVALUATION

This appendix contains case examples to illustrate the application of the framework for mode-of-action analysis. Evaluations of mode-of-action information will ordinarily appear before or within the hazard characterization section of a risk assessment. Since these examples are given outside of a risk assessment, the basic data that underlie the evaluation are summarized first for reference, followed by the mode-of-action analysis.

D.1.0. EXAMPLE 1: CHEMICAL T (*THYROID DISRUPTION*)

D.1.1. HAZARD DATA SUMMARY

D.1.1.1. Data Availability

Data include a rat chronic/carcinogenicity feeding study, an 18-month CD-1 mouse carcinogenicity study, a 1-year dog feeding study, a subchronic feeding study in the rat, a 4-week and 1-year subchronic feeding study in the dog, a 21-day dermal study in the rat, developmental toxicity studies in the rat and rabbit, a two-generation reproduction study in the rat, mutagenicity studies, metabolism studies, and special subchronic mechanistic studies.

D.1.1.1.1. *Rat*

D.1.1.1.1.1. *24-month toxicity.* Male and female Sprague-Dawley rats received chemical T in the diet for 24 months. Thyroid follicular cell tumor incidence was increased in male but not female animals (see Table D-1). Tumor incidence in the two high-dose male groups was higher than in historical control studies. Thyroid and liver weights were increased in the two high-dose groups. A few renal tubular adenomas occurred in dosed male and female animals, but there was no statistical significance. SGPT was increased in high-dose animals; some other liver enzymes were increased at various times.

Table D-1. Thyroid follicular cell tumor incidence in male rats

Tumor	Dose (ppm in diet)					
	0	**1**	**10**	**100**	**1000**	**3000** [a]
Benign	1/50 [b]	2/47	0/49	2/47	8/49	12/48 [b]
Malignant	1/50 [b]	1/47	0/49	0/47	1/49	4/48
Combined	2/50 [b]	3/47	0/49	2/47	9/49	14/48 [b]

[a]Two animals had both benign and malignant tumors.

[b]Statistically significant for trend noted at control; pairwise comparison noted at dose level.

D.1.1.1.1.2. ***Special subchronic studies*****.** Groups of male Sprague-Dawley rats were fed chemical T at 3000 ppm in the diet for 7, 14, 28, 56, or 90 days. Starting at 7 days, TSH levels were significantly increased and T_4 values were significantly decreased. There were also significant increases in thyroid and liver weights and for follicular cell hypertrophy and hyperplasia. Hepatic UDPGT activity for T_4 was increased, while hepatic 5'-monodeiodinase activity was either unaffected or decreased. Radioiodine uptake into the thyroid gland was measured. The percent of the dose per gram of thyroid tissue was equivalent in 3000 ppm and control groups, as was protein-bound iodide per mg of thyroid protein. Activities of hepatic aryl hydrocarbon hydroxylase, ethoxycoumarin O-dehydrase, and cytochrome P-450 were significantly increased in chemical T dosed animals.

Groups of male Sprague-Dawley rats were fed chemical T (30, 100, 300, 1000, 3000 ppm) for 56 days; some animals were taken off chemical T for another 56 or 112 days to evaluate reversibility of effects. Thyroid weights were significantly increased in the top two doses, while liver weights were increased in the top three doses. T_4 UDPGT activity was increased in the top two doses. T_4 was decreased and TSH increased at the top dose, along with increases in the incidence of follicular cell hypertrophy and hyperplasia. Upon stopping chemical T dosing, all parameters returned to normal except for thyroid weight. Elimination of radioiodine-labeled T_4 from the blood and into the bile was measured after 56 days of chemical T dosing. Blood clearance was twice as fast in dosed animals as in controls, while there was a 40% increase in the rate of excretion of the hormone into the bile of treated animals.

D.1.1.1.2. ***Dog***

D.1.1.1.2.1. ***Subchronic toxicity*****.** Subchronic feeding of chemical T (0, 10, 100, 1000, 5000 ppm) produced an increase in thyroid weight and hyperplasia of the gland at 5000 ppm. There was hepatocellular hypertrophy at 1000 ppm and above.

D.1.1.1.2.2. ***12-month toxicity*****.** One-year feeding of chemical T (1, 20, 200, 2000 ppm) led to hepatocellular hypertrophy/hyperplasia at 200 and 2000 ppm but not at 0 or 20 ppm. At 2000 ppm, absolute and relative liver weights were increased. At 2000 ppm, there were increases in SGOT, SGPT, GGT, and ALK, and decreases in cholesterol, albumin, and total protein.

D.1.1.1.3. ***Mouse***

D.1.1.1.3.1. ***18-month toxicity*****.** In an 18-month chemical T feeding study (0, 1, 10, 100, 400, 800 ppm), there were no increases in tumor incidence at any site. Absolute and relative liver

weights were statistically significantly increased over controls at the highest dose level, as were kidney weights in the female. Increases in liver enzymes were noted at various intervals, including SGPT, SGOT, and ALK. Dose levels in the study were considered adequate.

D.1.1.2. Mutagenicity

Negative results were seen in four strains of Salmonella with or without metabolic activation; negative results in assay of forward mutation of HGPT locus of Chinese hamster ovary cells (dosing probably not sufficient); negative results in mouse bone marrow micronucleus assay; negative results in assay for unscheduled DNA synthesis in rat hepatocytes pretreated with chemical T. The compound does not have a structure that suggests electrophilicity.

D.1.2. SUMMARY DESCRIPTION OF POSTULATED MODE OF ACTION

Thyroid hormone production is regulated by actions of the hypothalamus, pituitary, and thyroid glands. Homeostasis of thyroid hormone is maintained by a feedback loop among the hypothalamus and pituitary and the thyroid gland. The hypothalamus produces thyrotrophin reducing hormone (TRH), which stimulates the pituitary to produce thyroid stimulating hormone (TSH) which, in turn, stimulates the thyroid to produce thyroid hormone. The hypothalamus and pituitary respond to a high level of circulating thyroid hormone by suppressing TRH and TSH production, and to a low level by increasing them. The mode of action considered is continuous elevation of TSH levels that stimulates the thyroid gland to deplete its stores of thyroid hormone and continues to push production, resulting in hypertrophy of the production cells (follicular cells) leading to hyperplasia, nodular hyperplasia and, eventually, tumors of these cells. In rats, the chain of events may be induced by direct effects on hormone synthesis or by metabolic removal of circulating hormone.

D.1.3. KEY EVENTS

The key events considered with respect to chemical T-induced tumorigenesis in male rats include hormone changes in TSH, T_4, and T_3, and changes in hepatic T_4-UDPGT, indicators of liver microsomal enzyme induction, enhanced liver metabolism, increased biliary excretion of T_4, increase in thyroid weight and liver weight, and thyroid follicular cell hypertrophy/hyperplasia. These events have been well defined and measured in male rats in subchronic studies, augmenting observations at interim and terminal sacrifice in a chronic study.

D.1.4. STRENGTH, CONSISTENCY, SPECIFICITY OF ASSOCIATION OF TUMOR RESPONSE WITH KEY EVENTS

The thyroid tumor response in the chronic study at the highest dose was associated with hypertrophy/hyperplasia in the thyroid and increase in weight of the thyroid. In subchronic studies, the organ weight and hypertrophy/hyperplasia were shown to appear and reverse in statistically significant degrees under the same conditions of dose and time as the appearance and reversal of changes in thyroid hormone levels and thyroid hormone metabolism. Stop/recovery studies showed that cessation of dosing was followed in turn by return of hormone levels to control levels, reduction in liver and thyroid weights, and reversal of hyperplasia in thyroid follicular cells. The only sign slow to reverse was thyroid weight after the longest dosing period. Strength, consistency, and specificity of association were well established in the studies.

D.1.5. DOSE-RESPONSE RELATIONSHIP

Dose correlations exist for parameters in the chronic and subchronic studies for all of the relevant parameters. Thyroid follicular cell tumors, thyroid hypertrophy/hyperplasia, and increased thyroid and liver weight are noted at similar doses, usually at dietary levels of 1000 and 3000 ppm chemical T. Correspondingly in the subchronic study, at 3000 ppm T_4 is depressed while TSH is elevated. At 1000 and 3000 ppm, hepatic T_4-UDPGT activity is statistically significantly elevated, and there is an increase in biliary excretion of T_4 at 3000 ppm. The only parameter showing significant effect at a dose below 100 ppm chemical T was liver weight increase in a subchronic study at 300 ppm.

D.1.6. TEMPORAL ASSOCIATION

The chronic study, together with the three subchronic studies of key events observing effects after different durations at one dose, at multiple doses, and after recovery, shows events occurring in the following sequence: (1) increase in hepatic glucuronidation, de-iodination and excretion of T_4, as well as its elimination from the blood; (2) a rise in circulating TSH; (3) an increase in thyroid weight and thyroid follicular cell hypertrophy; (4) thyroid follicular cell hyperplasia; and (5) thyroid follicular cell tumors. The stop experiments indicate reversal of the thyroid and liver weight increases as well as reversal of hormone and other protein measures. While reversal of thyroid weight increase in the recovery study was less after a longer duration of treatment, hypertrophy/hyperplasia did reverse after the longer duration.

D.1.7. BIOLOGICAL PLAUSIBILITY AND COHERENCE OF THE DATABASE

Under EPA science policy (U.S. EPA, 1998a), determination of the antithyroid activity of a chemical requires empirical demonstration of five items: (1) increases in thyroid growth, (2) changes in thyroid and pituitary hormones, (3) location of the site(s) of antithyroid action, (4) dose-response correlations among various key precursor events and tumor incidence, and (5) reversibility of effects following treatment cessation. The database on chemical T documents all such information.

Thyroid tumorigenesis, particularly in the male rat, has been observed to be associated with exposure to a number of industrial chemicals, pesticides, and pharmaceuticals. A significant number of these appear to work in a manner similar to chemical T, by enhancing thyroid hormone metabolism and excretion by the liver.

Thyroid tumors did not appear in the female rats in the 2-year study. Thyroid hypertrophy and hyperplasia were observed in the females 6 months after their appearance in males. As is noted with other chemicals, the female rat is less sensitive to the effect of antithyroid chemicals regarding key events and tumor development. Hepatic enlargement and effects are noted in the mouse and dog studies, as they are in the rat. In addition, dogs receiving high doses of chemical T show enlargement of the thyroid gland.

D.1.8. OTHER MODES OF ACTION

Chemical T does not belong to a class of chemicals that is expected to generate reactive metabolites, and no related chemicals have been tested for carcinogenicity. Short-term studies demonstrate that the chemical does not increase gene mutations in Salmonella (Ames test) or cultured mammalian cells (maximal dosage may not have been reached), micronuclei in bone marrow cells, and unscheduled DNA synthesis in cultured cells. No other modes of action, apart from thyroid disruption, are described to account for the thyroid tumors.

Several sites of action were investigated as being the source of the antithyroid effects of chemical T. The chemical does not inhibit the entry of inorganic iodide into the thyroid (iodide pump) or block the organification and incorporation of iodide into thyroid hormone (thyroid peroxidase); likewise, it does not inhibit monodeiodinase, which blocks the conversion of T_4 to T_3.

Chemical T administration leads to renal adenomas in male and female rats; the response lacked statistical significance. The mode of action for the thyroid tumors does not account for the renal tumors. Assessment of the significance and mode of action of the renal tumors requires separate analysis.

D.1.9. CONCLUSION

The weight of evidence supports a conclusion that chemical T acts by inducing hepatic metabolism and biliary elimination of thyroid hormone, prompting increased production of TSH, which ultimately results in thyroid follicular cell neoplasia as postulated.

D.1.10. RELEVANCE OF THE MODE OF ACTION TO HUMANS

Relevance to humans

Chemical T affects the liver of rats, mice, and dogs, and the thyroid of rats and dogs. Given the breadth of responses, it is possible that humans may respond similarly. The subject of the relevance of an antithyroid mode of action for thyroid tumors is extensively covered in the Agency's policy for the assessment of this mode of action (U.S. EPA, 1998a). In summary the policy states:

> The role of thyroid-pituitary disruption in cancer development in humans is much less convincing than in animals. Iodide deficiency is associated with increases in thyroid cancer in some studies but not others. Similarly, an association between either inborn errors of metabolism affecting thyroid hormone output or autoimmune-related Graves' disease and cancer is suggested but not proved. It seems that TSH may at least play some permissive role in carcinogenesis in humans. Accordingly, one cannot qualitatively reject the animal model; it seems reasonable that it may serve as an indicator of a potential human thyroid cancer hazard. However, to the extent that humans are susceptible to the tumor-inducing effects of thyroid-pituitary disruption, and given that definitive human data are not available, it would appear that quantitatively humans are less sensitive than rodents in regard to developing cancer from perturbations in thyroid-pituitary status.

The measured key events and their effects, as well as effects of reversal of the events, are consistent with what is known about the regulation of thyroid hormone balance, and the postulated carcinogenic mode of action as summarized above.

Thyroid tumorigenesis, particularly in the male rat, has been observed to be associated with exposure to a number of pesticides and pharmaceuticals. A pattern of thyroid organ growth, frequently liver growth, thyroid hormone changes, or changes in hormone metabolism has been

seen with a large proportion of these compounds. Chemical T effects are parallel to these other cases.

Thyroid tumors did not appear in the female rats in the 2-year study. Thyrotrophy and hyperplasia were observed in the females with a 6-month lag after their appearance in the male. The female is apparently more tolerant of thyroid disruption; whether tumors would have been seen in the females if the 2-year study had been extended is uncertain.

Relevance to subpopulations

Thyroid hormones are regulated within rather narrow ranges, with normal adult human serum values often being given as T4--4 to 11 ug/dL and T3--80 to 180 ng/dL. TSH levels extend over a broader range--0.4 to 8 ug/ml, due to the incorporation in recent years of more sensitive laboratory methods that have extended the normal range to lower values (Ingbar & Woeber, 1981; Surks et al., 1990). The upper bound on normal TSH has not changed, and it is the one of import to considerations of antithyroid effects of chemicals. During development somewhat higher levels for each of the hormones are noted, with adult hormone values being reached beyond about 10 years of age (Nicholson and Pesce, 1992). Growth of the thyroid gland continues for the first 15 years of life, going from about 1 gram at birth to an adult size of about 17 grams (Fisher and Klein, 1981; Larsen, 1982). Early developmental inability to synthesize adequate thyroid hormone leads to altered physical and mental development (cretinism) (DiGeorge, 1992; Goldey et al., 1995) and is treatable. The control of normal thyroid growth during development is not totally known, although the increase in gland size may be independent of TSH stimulation (Logothetopoulus, 1963). Extended deviations in human thyroid hormone levels either above or below the normal range are associated with hyperthyroidism and hypothyroidism, respectively and are treated in the U.S. to restore balance.

Thyroid cancer is a rare condition in the U.S., occurring with an incidence of about 0.004% per year (Greenspan & Strewler, 1997). The incidence is predominantly in persons over 30, and increases in older persons; in children the incidence is at the 1 per million rate. Mortality rates per 100,000 are above zero only for those older than 35 (Ries et al., 1999).

It is recognized that the human thyroid is susceptible to ionizing radiation, the only verified human thyroid carcinogen. Children are known to be more sensitive than adults to the carcinogenic effects of radiation (NRC, 1990; IAEA, 1996). The nature and consequences of radiation have differences from thyroid disruption by inborn deficits or possible chemical influence that is not mutagenic. The major effect of ionizing radiation on the thyroid is thought to be due to mutation. Antithyroid effects can also be induced at elevated radiation doses due to cytotoxicity of follicular cells with resulting reduction in thyroid hormone and elevation of TSH. Mutagenic

chemicals, however, do not act totally like radiation: (a) X rays penetrate the body and target organs without having to be absorbed. Chemicals must be absorbed and distributed to target organs. (b) Unlike most organic chemicals, radioiodine is actively transported and concentrated in the thyroid gland, and it becomes incorporated into nascent thyroglobulin. (c) Given that the size of the thyroid gland is smaller in children than in adults, for a given blood level of radioiodine, the internal dose to the thyroid of a child is greater than that for an adult. (d) Radioiodine in the Chernobyl accident was picked up by cattle and incorporated into milk. Due to differences in milk consumption, the external dose presented to children was greater than to adults.(e) Single quanta of radiation result in a series of ionizations within biological material, each of which can react with DNA to induce mutations and affect the carcinogenic process. Chemicals are much less efficient: they frequently need to be metabolized to active intermediates, with each molecule interacting singly with DNA, usually by forming adducts which can be converted to mutations. (f) The spectrum of mutagenic effects vary with the source. Ionizing radiation often results in deletions and other structural chromosomal aberrations, while chemicals not uncommonly produce more gene mutations. (g) The thyroid of children is more sensitive to carcinogenic effects of external radiation on a per unit dose basis than in adults, especially for children less than 5 years of age. Sensitivity decreases with advancing age and seems to disappear in adulthood. It is estimated that, overall, children may be two or more times more sensitive to carcinogenic effects of external emitters than are adults (NRC, 1990).

The evidence supports the view that Chemical T's mode of action will not be different for children. Thyroid cancer is very rare in younger age groups and lower in incidence and mortality than for older adults. It does not appear that the young have any propensity for thyroid cancer from which one could infer some underlying cancer process that differs from adults (absent ionizing radiation treatment or incidents, discussed above). The basic elements of thyroid function and hormone homeostasis are the same in children and adults with a period of growth during which children reach lower adult balances. The chemical disruption mode of action of Chemical T in animals, to the extent that it is applicable to humans, appears equally applicable to human subpopulations. It is not expected to share the features of radiation.

D.2.0. EXAMPLE 2: CHEMICAL Z (BLADDER TUMOR)

D.2.1. HAZARD DATA SUMMARY

D.2.1.1. Data Availability

Data include a rat chronic/carcinogenicity feeding study, an 18 month CD-1 mouse carcinogenicity study, a three-generation reproduction study in the rat, and a 2-year feeding study in dogs. There are no data on the effects in humans of exposure to chemical Z.

A 13-week feeding study in rats included interim sacrifices at 2, 4, and 8 weeks and establishment of 16-week recovery groups at 8 weeks and a 21-week recovery group at 13 weeks.

D.2.1.2. Tumor Observations

D.2.1.2.1. *Tumor Response*

D.2.1.2.1.1. *Rats.* Administration of chemical Z in the diet to male Sprague-Dawley rats at dose levels of 30,000 ppm or more for 2 years resulted in an increase in bladder urothelial tumors in male rats. Statistically significant increases ($p<0.05$) were noted at the high dose only (40,000/30,000 ppm) in the incidences of transitional cell papillomas, carcinomas, combined papillomas and carcinomas, and hyperplasia in the 2-year SD rat bioassay (Table D-2). Bladder calculi were observed in some animals but correlation between stones and tumors was not evident at final sacrifice.

Table D-2. Incidence of transitional cell lesions and stones in the bladder of males from a 2-year SD rat study

Parameter	Dose (ppm)			
	0	**2000**	**8000**	**40,000/30,000**
N	73	75	78	78
Lesion				
Papilloma	1	1	1	5
Carcinoma	2	2	1	16
Combined	3	3	2	21
Hyperplasia	5	7	5	29
Stones	0	0	0	5

D.2.1.2.1.2. ***Mice.*** No increase in tumor incidences was observed in an 18-month bioassay with mice.

D.2.1.3. Mutagenicity

Chemical Z has not shown mutagenic activity based on results of *Salmonella sp.* or micronucleus assays. No evidence exists that the chemical produces effects on DNA synthesis nor does it appear to be clastogenic. There are no structural alerts suggesting mutagenic potential for the chemical.

D.2.1.4. Toxicity, Uroliths, and Hyperplasia

There was a strong association among disruptions in urinary physiology, toxicity, uroliths, and hyperplasia in the 13-week study in mid-dose and high-dose animals (30,000 and 50,000 ppm respectively, [$p<.05$]). In the control and 8,000 ppm group, no animals had stones and no animals had hyperplasia (see Table D-3).

Table D-3. Incidence of bladder hyperplasia and stones in male SD rats treated up to 13 weeks

Parameter	2 weeks				8 weeks				13 weeks			
Dose[a]	1	2	3	4	1	2	3	4	1	2	3	4
N	10	10	10	10	10	10	10	9	10	10	10	6
Papillary hyperplasia	0	0	7	8	0	0	9	7	0	0	5	6
Simple hyperplasia									0	0	2	0
Stones	0	0	3	4	0	0	9	8	0	0	7	6

[a]Dose (ppm): 1 = control, 2 = 8000, 3 = 30,000, 4 = 50,000.

D.2.1.4.1. *Thirteen-Week Study*

Urothelial toxicity and disruptions in urinary physiology and urothelial toxicity appeared early in the study. Early changes in urinary physiology (decreased pH and increased cation concentration) were observed following 2 weeks of treatment and persisted throughout the duration of the study. Urothelial toxicity was expressed as edema, cystitis, and hyperplasia; hyperplasia (simple and papillary transitional cell combined) increased in overall incidence with continued treatment. It was present in 70% of mid-dose (30,000 ppm) animals and 80% of high-dose (50,000 ppm) animals following 2 weeks of exposure, and in 70% of the mid-dose group and 100% of the high-dose group at 13 weeks. There was some indication of a decrease in severity of hyperplasia at 13 weeks when compared to earlier time periods, as there was an apparent shift from the incidence of papillary hyperplasia to simple hyperplasia and a decrease in the combined incidence of hyperplasia in the 30,000 ppm group of animals.

Uroliths were found to be present as early as 2 weeks (0%, 0%, 30%, and 40%) and the incidence increased over the period of the study. The incidence of uroliths at termination of the 13-week study was 0%, 0%, 70%, and 100%, but there was a decrease in size and number of stones per animal at 13 weeks.

D.2.1.4.2. *Three-Generation Reproduction Study in Rats*

High dose levels (>20,000 ppm in the diet) led to formation of lesions in the urinary tract of males and females of the F1, F2, and F3 generations. The lesions included hemorrhage of the bladder wall, increased pelvic dilation, and papillary necrosis. In the F3 generation, additional effects noted in renal tissue were hyperplasia of the transitional epithelium and desquamation of cells in the lumen of the urinary tract. The changes were associated with crystalline or calcareous deposits.

D.2.1.5. Reversibility of Effects

There was strong evidence of reversibility of bladder stones and bladder hyperplasia. When animals that had been treated for 8 weeks were returned to basal diet for 16 weeks, uroliths were found in 30% of 30,000 ppm animals and 25% of high-dose animals. Bladder hyperplasia (papillary and transitional cell combined) was reduced to 25% and 30% in each of these two dose groups (Table D-4). An analysis of individual animal data revealed a strong correlation between the incidence of uroliths and hyperplasia at the termination of the recovery period.

Table D-4. Reversal of incidence of bladder hyperplasia and stones following 8 weeks treatment and 16 weeks recovery

Parameter	Dose (ppm)			
	0	**8000**	**30,000**	**50,000**
N	10	10	10	8
Papillary hyperplasia	0	0	2	1
Simple hyperplasia	0	0	1	1
Stones	0	0	3	2

D.2.1.6. Blood and Urine Chemistry

Chemical Z administration resulted in increases in blood phosphorus and carbon dioxide (data not shown). Urinalyses (Table D-5) showed elevated calcium levels, reduced urinary phosphorus, and a profound lowering of urinary pH (5.0), which began at 2 weeks and persisted throughout the 13-week study in the 30,000 and 50,000 ppm group of rats. These changes occurred in the presence of bladder stones, which were reported to consist of 33% calcium and 23% phosphorus.

Table D-5. Clinical chemistry values (urine) in male SD rats treated up to 13 weeks

Parameter	2 weeks				8 weeks				13 weeks			
Dose	1	2	3	4	1	2	3	4	1	2	3	4
N	10	10	10	10	10	10	10	9	10	10	10	6
Calcium - mg/dL	6	11	56[b]	36[c]	11	11	18	65[b]	5	7	14[b]	58[b]
Phosphorus - mg/dL	90	62	2[b]	13[c]	109	90	19	1[b]	57	67	26	1[b]
pH	7	6.5	5[b]	5[b]	7.4	6.9	5.8[b]	5.0[b]	7.2	6.7	6.0[b]	5.0[b]
Stones	0	0	3	4	0	0	9	8	0	0	7	6

[a]Dose (ppm): 1 = control, 2 = 8000, 3 = 30,000, 4 = 50,000.
[b]$p<.01$.
[c]$p<.05$

D.2.1.7. Metabolism

Upon ingestion by rats, the ethyl moiety of chemical Z is rapidly absorbed, hydrolyzed to a phosphite, and oxidized via acetaldehyde and acetate to carbon dioxide and water. Absorption of the phosphite moiety leads to increased blood phosphorus levels. There is also an increase in blood calcium load, which leads to increased excretion of calcium via the urine. Ethyl phosphite moieties and carbon dioxide are also eliminated via the urine. A marked depression of urinary pH (5.0) results from acidification of the urine by carbon dioxide. An aluminum moiety of the parent chemical is poorly absorbed, and most is eliminated in the feces. The phosphite metabolite, the major urinary metabolite, was not shown to express carcinogenic potential when administered to Sprague-Dawley rats at dose levels up to 32,000 ppm. It also does not express any mutagenic potential and does not have any structural alerts.

D.2.1.8. Structure-Activity Relationships

There are no data on structurally related chemicals.

D.2.2. MODE-OF-ACTION ANALYSIS

D.2.2.1. Summary Description of Postulated Mode of Action

Chemical Z produces transitional cell tumors in male Sprague-Dawley rats. The mode of action includes disruption in urinary physiology, including precipitation of calcium and phosphorus and formation of bladder calculi. The stones irritate the urothelium of the bladder, followed by transitional cell hyperplasia and bladder tumor formation. Disruption of urinary physiology is a consequence of a metabolic sequence involving (1) absorption and metabolism of the ethyl moiety to carbon dioxide, resulting in a reduction in urinary pH; and (2) absorbtion of the phosphite moiety, which leads to increased blood phosphorus levels and increased release of calcium into the urine. Increases in water consumption followed by increased urinary volume may contribute to bladder toxicity, but a precise role of increased urinary volume has not been established.

The mode of action for chemical Z is consistent with other data that demonstrate that solid masses in the rodent bladder, regardless of their origin--insertion of solid materials, including inert pellets, precipitation of administered chemicals (e.g., melamine) or disruption of urinary physiology (e.g., diethylene glycol)--lead to urothelial toxicity and the formation of tumors.

D.2.2.2. Key Events

The key precursor events associated with bladder tumor formation following administration of chemical Z to rats include increased blood phosphorus and carbon dioxide, elevated urinary calcium and volume, decreased urinary pH and phosphorus, formation of bladder stones, and irritation and hyperplasia of the urothelium.

D.2.2.3. Strength, Consistency, and Specificity of Association of Tumor Response with Key Events

The only tumor response seen in animal studies is bladder tumors in male Sprague-Dawley rats. Studies in dogs and mice showed no effect on the bladder. The rat tumor response was seen only at high doses that lead to key precursor effects: altered urinary physiology (volume, calcium, pH) results in stones and produces toxicity and hyperplasia of the urothelium. The high-dose changes were noted in a rat chronic, a rat subchronic, and a three-generation reproduction study in rats. The key events, including hyperplasia, were observed to be reversible in subchronic stop/recovery studies. Administration of the major metabolite of chemical Z, monosodium phosphite, fails to reduce urinary pH, increase urinary volume, or produce nonneoplastic or neoplastic lesions of the bladder. The database on chemical Z is sufficient to evaluate the proposed mode of action despite the absence of more complete information on the composition of the stones and questions regarding the absence of toxicity following the administration of monosodium phosphite. There is a high degree of confidence that the findings accurately reflect the effects associated with administration of the chemical. No data gaps were identified that would substantially alter the evaluation of the proposed mode of action.

D.2.2.4. Dose-Response (D/R) Relationships

The 2-year bioassay showed urothelial hyperplasia, transitional cell papillomas, and transitional cell carcinomas and a few bladder stones at 40,000/30,000 ppm. Of 78 high-dose animals, 37% showed bladder tumors. Tumors, hyperplasia, and stones were not increased at 8000 ppm. A special 13-week feeding study demonstrated that key events--increased urinary calcium levels, decreased urinary phosphorus levels, decreased pH, bladder stones, irritation, edema, and hyperplasia--occurred consistently only at dose levels of 30,000 ppm or greater. A strong dose-response correlation was shown between calculus formation and hypercalciuria, acidic urine, and bladder hyperplasia. In a rat reproduction study, bladder effects were noted at 24,000 ppm but not at 12,000 ppm.

D.2.2.5. Temporal Association

A subchronic rat study with serial sacrifices at 2, 4, 8, and 13 weeks, including evaluation of 16-week recovery groups after 8 weeks and a 21-week recovery group after 13 weeks, was performed. By 2 weeks of administration, chemical Z produced stones that filled the bladder and resulted in advanced papillary hyperplasia. The number and size of stones was greatest at two weeks and there was a progressive decrease over the 13 week period. Early changes in urinary physiology (decreased urinary pH, increased calcium concentration, and decreased phosphorus concentration) were observed following 2 weeks of treatment and persisted throughout the duration of the study. Observation of the 8-week treatment/16-week recovery groups showed that incidence of both stones and hyperplasia significantly decreased as compared with incidence in animals sacrificed at 8 weeks. Also, upon cessation of dosing at 13 weeks, the incidence of animals with stones, the incidence of papillary hyperplasia, and the severity of hyperplasia decreased significantly by the end of a 21-week recovery period (data not shown). The changes noted within 2 weeks of dosing appear to have set in motion a series of events beginning with increased urinary calcium concentrations, followed or accompanied by stone formation, irritation of the bladder urothelium, hyperplasia and, eventually, neoplasia.

D.2.2.6. Biological Plausibility and Coherence of the Database

Long-term and subchronic studies with chemical Z have demonstrated a dose correlation between development of stones and bladder tumor formation in male rats. Data from the 13-week study indicate a rapid onset of effects (changes in urinary parameters, formation of stones, and hyperplasia within 2 weeks of dosing) and adaptation of treated animals to chemical Z exposure by 13 weeks (decreased numbers and size of stones per animal, decreased severity of hyperplasia). Tumors were observed only at doses at which key events were observed.

Additional bioassay data provide support for the association of tumors in rats with the key events in rats and the absence of both tumors and similar key events in other species treated with chemical Z. Treatment of rats in a three-generation reproduction study at high dose levels (>20,000 ppm in the diet) led to formation of lesions in the urinary tract of males and females. When administered to dogs at dose levels up to 40,000 ppm in the diet for up to 2 years, the chemical produced minimal toxic effects overall, no effects on the urinary tract, and no tumors. Chemical Z produced no effects in mice when administered up to a dose level of 20,000/30,000 ppm in the diet for 2 years.

Observations with chemical Z are in keeping with those observed in many other experimental settings. Stones, regardless of their chemical makeup, are irritating to the rodent bladder, causing irritation, hyperplasia, and eventually neoplasia.

There are some uncertainties regarding the role of certain findings following chemical Z administration. Generally, an increase in urinary pH is associated with the precipitation of calcium and phosphorus-containing stones in rats. However, stones are formed in the presence of a low urinary pH in rats administered chemical Z. It is also unclear whether or not the acidic environment of the urine (most likely a consequence of the conversion of the ethyl moiety to carbon dioxide in the blood) contributes to or enhances any effects noted in bladder tissue in rats. There was a paucity of stones in high-dose animals at termination of the 2-year study but a higher incidence of bladder tumors, which suggests that bladder stones may not be the causative factor involved in bladder tumor formation. Other considerations discount this presumption. First, a number of the high-dose animals showed hydronephrosis or dilation of the ureters, presumptive indications of past urinary tract obstruction. Second, the 13-week study provided evidence that bladder calculi develop rapidly (within 2 weeks), but then decreased in frequency and size. The decrease in size and number of bladder calculi was accompanied by a decrease in severity of bladder hyperplasia in animals treated with 30,000 ppm of chemical Z. Third, it is recognized that a constant ppm of an agent in the diet results in a reduction in dose per unit body weight as an animal grows. Finally, the increased urinary volume or decreased urinary pH may have led to a dissolution of stones over time.

The absence of bladder stones and urothelial toxicity following administration of the major metabolite, monosodium phosphite, is puzzling, as one might expect administration to rats would lead to similar bladder effects as with chemical Z. However, the metabolite when administered to rats, leads to an increase in blood levels of phosphorus but does not alter urinary volume or pH as would be expected with an increase in sodium consumption. Considering the high dose-level of metabolite administered to rats (32,000 ppm), it is unlikely an additional bioassay using higher dose-levels would provide useful information.

D.2.2.7. Other Modes of Action

Chemical Z is not mutagenic in short-term tests and it does not have a structure suggesting biological reactivity. No other modes of action, apart from that postulated, are in evidence. The fact that bladder tumors were the sole tumors seen in rats and that no other species showed tumors or other toxicities like those in the rat make it less likely that the agent has another generalized mode of action.

D.2.2.8. Conclusion

The available bioassay data on chemical Z are sufficient to support the postulated mode of action that the chemical, which lacks mutagenic potential, leads to bladder tumor formation in male rats through a sequence of key events involving perturbations in urinary physiology, especially increased calcium concentration, calculus formation, urothelial irritation, hyperplasia, and neoplasia.

D.2.3. RELEVANCE OF THE MODE OF ACTION TO HUMANS

Bacterial infection, urinary stones or a combination of the two may be risk factors for human urinary tract cancer (Burin et al., 1995; Davis et al., 1984; Gonzalez et al., 1991; Kawai et al., 1994; Hiatt et al., 1982). Infection of the bladder with *Schistosoma haematobium* leads to bladder tumors, and part of its action may be associated with stone formation (IARC, 1995). A significant relationship has also been shown between spinal cord injury and bladder cancer; chronic infection and stones are found in individuals so affected (Bickel et al., 1991; Broecker et al., 1981; Dolin et al., 1994; El-Marsi and Fellows, 1981; Stonehill et al., 1996). Case control epidemiologic studies (relative risks less than three) suggest associations between bladder cancer and urinary tract stones (Burin et al., 1995; Gonzalez et al. 1991). A large cohort study supports the association shown between bladder stones and bladder cancer (Chow et al., 1991). Taken as a whole, stones may play some role (particularly, along with infection) in bladder cancer formation. Bladder cancer is a disease of advancing age, with about 2/3 of all cases occurring among persons aged 65 years or older (Hankey et al., 1993).

Stones occur much more frequently in the upper urinary tract than in the bladder of humans (about 10% of urinary stones are found in the bladder), presumably because the upright posture of humans predisposes them to expelling stones through the urethra once a stone passes from the kidney to the bladder (Hiatt et al., 1982; Johnson et al. 1979; DeSesso, 1995). This characteristic, as well as the pain which accompanies such stones and leads to their surgical removal. Stones in the rodent bladder tend to be retained, because of their horizontal position. These findings suggest suggest that there may be a lower susceptibility of humans compared to rodents to the development of urinary tract tumors associated with stones.

Precipitation of chemicals in the urinary tract with the formation of stones is a common finding, with about 12% of males and 5% of females having a history over a lifetime of at least one stone (Johnson et al., 1979). Compared to adults, urinary stone formation in children is an

uncommon occurrence except in individuals with a predisposing condition, such as, various inborn errors of metabolism (e.g., cystinuria) and congenital malformations (Gearhart et al., 1991). The prevalence of urinary stones in children is about 1 case per 20,000 per year (0.005%) (Khoory et al., 1998). Only about 5% of stones are initially manifest during the first 20 years of life (Johnson et al., 1979). Causes of urinary stones in children are remarkably similar to those of adults (Khoory et al., 1998; Stapleton, 1996). Like with adults, the urine of children varies in pH and osmolality, particularly in response to diet and physiologic stressors (e.g., exercise, heat). Urinary excretion of chemicals occurs throughout life, although there may be quantitative differences associated with a number of factors including disease states and nutritional status. Stones used to be more common in children in developed countries than they are now, largely due to malnutrition, which is still a problem in developing nations today (Trinchieri, 1996).

Chemical Z is converted to metabolic derivatives through simple hydrolysis, a chemical conversion that does not depend on enzymatic activity. It is not plausible that differences in levels of enzymatic activity, such as detoxification via hepatic metabolism or metabolism in other tissues will alter, qualitatively, responses in population subgroups such as the aged, the infirm, or infants and children who may be exposed to Chemical Z.

In summary, the potential human carcinogenic hazard of the chemical cannot be dismissed for Chemical Z. Chemical Z poses a carcinogenic hazard to humans only under conditions that would lead to the formation of bladder stones. It is reasonable to conclude that the mode of action involving stone formation for Chemical Z that has been developed for adult animals may be applicable to young animals and to children. Information suggests that effects in the young may not be any greater than in adults and, in fact, the young may be less susceptible unless there are rare extenuating factors.

3.0. EXAMPLE 3: CHEMICAL D

D.3.1. HAZARD DATA SUMMARY

D.3.1.1. Data Availability

Human data are inadequate to establish a basis for carcinogenicity. Experimental data include:

- Three chronic toxicity and carcinogenicity studies in rats and mice: an inhalation study, an oral dietary study, and an oral gavage study;
- Subchronic studies by the oral and inhalation routes in rats and mice;
- Inhalation developmental toxicity studies in rats and rabbits;
- An inhalation two-generation reproductive toxicity study in the rat;
- In vitro and in vivo genotoxicity studies;
- Toxicokinetic and metabolism studies; and
- Protein binding studies.

D.3.1.2. Carcinogenicity/Chronic Toxicity

Chemical D has been shown to cause increased tumor incidences in rats and mice. The tumor responses seem to be dependent on the tested animal species, sex, dose, and route of administration. Results of available chronic bioassays are summarized in Table D-6.

Table D-6. Summary results of chronic bioassays

Study/dose	F344 rats	B6C3F1 mice
Oral gavage *Rat study*: 0, 25, 50 mg/kg (5 d/wk for 2 yr) *Mouse study*: 0, 50, 100 mg/kg (5 d/wk for 2 yr)	**Forestomach:** Papillomas (males: 1/50, 2/50, 8/50; females: 0/50, 2/50, 3/50) Carcinomas (males only- 0/50, 0,50, 4/50) Basal cell and epithelial hyperplasia (dose-related; males and females) **Liver:** Adenomas (males:1/50, 6/50, 7/50) Carcinomas (males: 0/50, 1/50, 3/50)	**Forestomach:** Papillomas (males: 0/50, 1/50, 5/49; females: 0/50, 2/50, 7/50) Carcinomas (females only: 0/50,1/50, 4/50) Basal cell and epithelial hyperplasia (dose-related; males and females) **Lung:** Adenomas (males: 2/50, 4/50, 8/49; females: 2/50, 4/50, 7/50) Carcinomas (males only: 0/52, 2/52, 4/49)
Oral dietary *Rat study*: 0, 2.5, 12.5, 25 mg/kg/day for 2 yr *Mouse study*: 0, 2.5. 25, 50 mg/kg/day for 2 yr	**Forestomach:** Basal cell and epithelial hyperplasia (dose-related; males and females) **Liver:** Adenomas (significant in males only: 2/50, 1/50, 6/50, 9/50)	No histopathologic changes
Inhalation *Rat study*: 0, 5, 20, 60 ppm (6 hr/d 5 d/wk for 2yr) *Mouse study*: 0, 5, 20, 60 ppm (6 hr/d 5 d/wk for 2yr)	**Nasal cavity:** Epithelial hyperplasia (dose-related; males and females)	**Nasal cavity:** Epithelial hyperplasia (dose-related; males and females) **Lung:** Adenomas (males only: 2/50, 3/50, 6/50)

- In rats, chemical D caused dose-related increases in liver tumors (males only) and forestomach tumors (both sexes) via oral gavage, but only liver tumors (males high dose only) by ingestion. No tumors were found in an inhalation study.
- In mice, chemical D caused dose-related increases in forestomach and lung tumors (both sexes) by oral gavage, but no tumors were observed in the oral dietary study. Chemical D only induced an increased incidence of lung tumors in male mice exposed to the high dose by inhalation.
- Nonneoplastic changes were observed in the forestomach of treated rats (gavage and dietary studies) and mice (gavage only) of both sexes. Chemical D also induced nonneoplastic changes in the nasal mucosa of rats and mice of both sexes via inhalation.

D.3.1.3. Subchronic Toxicity

Subchronic toxicity studies have been conducted in rats and mice by the oral and inhalation routes. The primary organs affected were the forestomach (rats) and the liver (mice) via oral exposure, and the nasal cavity and respiratory tract of both rodent species via inhalation.

D.3.1.3.1. *Oral Studies*

Groups of F344 rats (10 animals of each sex per dose group) were administered 0, 5, 15, 50, or 100 mg/kg/day of chemical D via their diets for 13 weeks. Dose-related decreases in body weight gain were observed in treated males and females. Basal cell hyperplasia and hyperkeratosis of the forestomach was found in males and females rats treated with chemical D at the three highest doses.

B6C3F1 mice (10 animals of each sex per dose group) were administered 0, 25, 50, 100, or 175 mg/kg/day via their diets for 13 weeks. Body weight gains of treated males and females were depressed in a dose-related manner compared to controls. Histologic changes were noted in the liver and were characterized as decreased hepatocyte size in all treatment groups. This observation was consistent with decreased hepatocellular cytoplasmic glycogen.

D.3.1.3.2. *Inhalation Studies*

F344 rats (10 animals of each sex per dose group) were exposed to 0, 10, 30, 90, or 150 ppm of chemical D for 6 hr/day, 5 days/week for 13 weeks. Treatment-related effects included depressed body weight gain (at 30 ppm and greater), degenerative changes in nasal olfactory

epithelium, and hyperplasia of respiratory epithelium in both males and females (at 90 and 150 ppm).

B6C3F1 mice (10 animals of each sex per dose group) were exposed to 0, 10, 30, 90, or 150 ppm of chemical D for 6 hr/day, 5 days/week for 13 weeks. Treatment-related effects included depressed body weight gain (at 30 ppm and greater), and histopathologic changes in the respiratory and olfactory epithelium of the nasal mucosa of both sexes exposed to 30, 90, and 150 ppm).

D.3.1.4. Developmental and Reproductive Toxicity

Pregnant F344 rats and New Zealand White rabbits were exposed to 0, 20, 60, or 120 ppm of chemical D during gestation days 6-15 (rats) and 6-18 (rabbits). Maternal effects (decrease body weight gain) were observed in rabbits and rats, in all treatment groups. A slight but statistically significant increase in the incidence of delayed ossification of the vertebral centra was observed in rats exposed to the high dose level. No developmental effects were observed in the rabbit study.

Exposure of F344 rats to 0, 10, 30, or 90 ppm of chemical D for up to two generations did not induce any effects on reproductive parameters or neonatal growth and survival in any of the generations. Parental effects were limited to epithelial degeneration of the nasal mucosa of the adults rats exposed to 90 ppm.

D.3.1.5. Mutagenicity

Chemical D was tested in many assays for gene mutation and chromosomal aberrations, as well as assays indicative of DNA damage, DNA strand breaks, and DNA alkylation. A heterogeneous database is found (a few in vitro positive responses and several negative results). It has been suggested that this heterogeneity is due to different studies that have used different test materials containing varying levels of impurities.

A few studies demonstrated that chemical D was weakly positive in the Ames bacterial assays in the presence of liver microsomes. Addition of cytosolic enzymes, presumably containing the detoxification enzyme glutathione transferase (GST), abolished mutagenic activity. Studies for chromosomal aberrations in vitro assays using mammalian cells have tended to be negative. There are a few positive results reported, but these are inconsistent with negative studies conducted in the same assay.

There are very few in vivo genotoxicity studies on chemical D. Chemical D has been found to be negative in a mouse micronucleus assay when tested up to oral doses of 175 mg/kg.

Chemical D has been reported to produce sister chromatid exchanges (SCEs) in mice. It should be noted that this assay has a low specificity for predicting carcinogenesis (i.e., a high rate of false positives compared to results of the rodent cancer bioassay). No dominant lethal effects (i.e., germ cell genetic damage) were found in rats exposed to chemical D by inhalation up to 150 ppm.

In vivo DNA binding studies were conducted in rats and mice. Rats were exposed acutely to chemical D at doses of 0, 10, 25, or 100 mg/kg. Mice were exposed acutely by inhalation to chemical D at 0, 30, and 60 ppm. No significant DNA binding (as measured by ^{32}P postlabeling assay) was seen in liver tissue from treated rats and lung tissue from exposed mice. In the mouse, DNA strand breakage was also studied by alkaline elution. Negative results were reported.

D.3.1.6. Toxicokinetic and Metabolism Studies

Toxicokinetic and metabolism studies in rats and mice have demonstrated that chemical D was rapidly absorbed by the oral and inhalation route. Blood half-lives were less than 10 minutes. Mercapturic acid conjugate of chemical D was the only major metabolite detected in the urine of treated rats and mice (about 80-90% of administered dose). Conjugated metabolites of chemical D epoxide were not detected in the urine of treated rats and mice.

Significant dose-related decreases in hepatic and lung tissues of GSH were observed in rats treated acutely with chemical D at oral doses of 0, 5, 20, 50, or 100 mg/kg, and in mice exposed acutely by inhalation to 0, 30, 60, or 150 ppm, respectively.

D.3.1.7. Protein Binding Studies

Chemical D was found to bind with tissue proteins in the forestomach and liver of rats treated acutely with oral doses 10, 50, and 100 mg/kg. Chemical D binding to tissue proteins was also found in the lung of mice exposed via acute inhalation at 30, 60, or 100 ppm.

D.3.2. MODE-OF-ACTION ANALYSIS

D.3.2.1. Summary Description of Postulated Mode of Action

It is postulated that chemical D causes tumors in rats and mice only when it is administered at high doses and/or by bolus administration that overwhelms the detoxifying mechanisms. The tumorigenic responses also appear to be closely associated with tissue toxicity (e.g., rat and mouse forestomach) and high background spontaneous tumors (e.g., mouse lung, rat liver). These observations, coupled with the lack of significant in vivo mutagenic activity, lead to the postulation that chemical D-induced tumorigenicity is likely to be operated by a nonmutagenic

mode of action, and appears to be secondary to toxicity and reparative cell proliferation. At high doses, a mutagenic mode of action may also be involved.

It is postulated that once absorbed, chemical D is biotransformed spontaneously or by microsomal mixed functional oxidases (MFO) to an epoxide derivative that can react directly with DNA. Both parent chemical D and the epoxide derivative are rapidly conjugated with glutathione (GSH), which then can be excreted in the urine, mainly as the mercapturic acid conjugate of chemical D. Under normal physiologic conditions, i.e., at nonsaturating doses, chemical D is effectively detoxified as glutathione conjugate, and epoxidation does not take place in any great extent. At high doses, chemical D is expected to react chemically with thiols in proteins, causing tissue toxicity (forestomach), depleting tissue GSH, and causing proliferation of high background spontaneous foci of altered cells (rat liver and mouse lung) leading to tumorigenesis. As less GSH is available for detoxifying chemical D, more chemical D is metabolized to the mutagenic epoxide derivative, which may play a role in the carcinogenic process.

D.3.2.2. Key Events

D.3.2.2.1. *Metabolism*

It is hypothesized that epoxidation of chemical D does not take place to any great extent since conjugated metabolite(s) of chemical D epoxide have not been detected in the urine of treated rats and mice. This finding was based only on acute exposure to chemical D. The metabolic profile of chemical D might differ under repeated exposures, particularly because chemical D has been found to deplete tissue GSH. Additional in vitro and in vivo metabolism studies are needed to further elucidate the potential role of MFO and epoxidation of chemical D.

D.3.2.2.2. *Tissue Toxicity*

It is postulated that chemical D-induced tumorigenicity is secondary to toxicity. The only target organ that exhibits both toxicity and tumorigenicity is the forestomach of rats and mice. Liver and lung toxicities have not been observed in chronic studies, although they have been reported in subchronic studies at higher doses. On the other hand, nasal toxicity was observed in exposed rats and mice, but no tumors were found.

Furthermore, the data supporting the postulated mechanism(s) of chemical D-induced toxicity are limited. It is hypothesized to be mediated by chemical D binding to tissue proteins. The only available information is the finding from acute oral and inhalation studies showing dose-related chemical D binding to proteins of the liver and forestomach of rats, and lung of mice,

respectively. Additional studies are needed to investigate the potential toxicity of chemical D at the biochemical, molecular, cellular, and tissue levels.

D.3.2.2.3. *Depletion of GSH*

The ability of chemical D to deplete tissue GSH has been demonstrated to take place only in the liver and forestomach of rats following acute ingestion and in the lung of mice via acute inhalation. Additional data are needed to examine the effects of chemical D on GSH levels in target organs as well as unaffected organs after repeated exposure.

D.3.2.2.4. *Proliferation Activity*

There is no information to substantiate the postulate that chemical D promotes highly spontaneous rat liver or mouse lung altered cells. Cell proliferation and mutation spectra studies are needed to examine the proliferative potential of chemical D.

D.3.2.3. Strength, Consistency, Specificity of Association of Tumor Response With Key Events

As discussed above, the postulated key events have not been clearly established. Thus, it is difficult to determine how well these key events relate to the observed tumorigenic responses. In general, the relationship between toxic and carcinogenic effects of chemical D on the forestomach of rats and mice is relatively stronger and more consistent than its effects on the rat liver and the mouse lung.

D.3.2.3.1. *Forestomach Tumors*

Subchronic studies and chronic studies in rats and mice demonstrate that the forestomach is the primary target by oral exposure to chemical D. The rat appears to be more susceptible to chemical D-induced forestomach toxicity than the mouse.

Dose-related neoplastic and nonneoplastic lesions of the forestomach were observed in treated rats and mice of both sexes when chemical D was administered by gavage. In contrast, only hyperplastic lesions of the forestomach were found in male and female rats following subchronic and chronic dietary exposures to chemical D. No histopathologic changes were observed in the forestomach of treated mice in the subchronic and chronic dietary studies.

D.3.2.3.2. *Liver Tumors*

Chronic exposure of chemical D caused increased incidences of hepatic adenomas in male rats when administered in the diet and by gavage. However, nonneoplastic changes in the liver were not observed in male rats after chronic or subchronic oral exposure to chemical D.

D.3.2.3.3. *Lung Tumors*

Chemical D induced increased incidences of lung adenomas in exposed mice via chronic inhalation (males only) and oral gavage. Nonneoplastic changes in the lung of exposed mice were not reported in the chronic study.

D.3.2.4. Dose-Response Relationships

As discussed above, dose correlations were demonstrated for chemical D-induced toxicity and/or carcinogenicity in the various target tissues of treated rats and mice. Dose-related depletion of tissue GSH was demonstrated with chemical D. However, no dose-related data are available for other toxicokinetic and metabolic parameters (absorption, uptake, distribution, metabolism, clearance and excretion of chemical D and metabolites), in vivo DNA binding, and other key events (e.g., cytotoxicity, cell proliferation) that are postulated to be involved in the tumorigenic process.

D.3.2.5. Temporal Association

While there are limited data indicating an association between chemical D-induced carcinogenicity and related toxicity (mostly for the forestomach), there are no data to discern the temporal association of these effects. Moreover, no data are available to establish the sequence of key events at the biochemical, molecular, or cellular levels that might mediate the tumorigenic responses.

D.3.2.6. Biological Plausibility and Coherence of the Database

The postulated mode of action for chemical D-induced forestomach tumors in rats and mice appears plausible and coherent with current knowledge. Many chemicals that are strong irritants have been shown to cause forestomach tumors via bolus administration. Similarly, the mouse lung appears to be more susceptible to the carcinogenic actions of many toxicants by inhalation. On the other hand, the observation that chemical D induces liver tumors only in the rat is not consistent with the general observation that the mouse is more susceptible than the rat to the carcinogenic effects of many halogenated hydrocarbons.

D.3.2.7. Other Modes of Action

Chemical D bears a structural resemblance to several short-chain halogenated hydrocarbons that are also animal carcinogens. Chemical D is expected to generate a mutagenic epoxide. Chemical D has been shown to exhibit weak mutagenic responses in a number of in vitro bacterial assays in the presence of liver microsomes, although addition of cytosolic enzymes, presumably containing GST, has been shown to abolish the mutagenic activity. Several cytogenetic assays demonstrated that chemical D can cause chromosomal damage in mammalian cells. Thus, a mutagenic mode of action cannot be entirely ruled out for chemical D.

D.3.2.8. Conclusion

There is little evidence to support a conclusion that chemical D-induced tumorigenicity in rats and mice is mediated by a nonlinear mode of action. The key events responsible for the tumorigenic responses are not well defined and a temporal association of these key events has not been fully investigated. Furthermore, it is still not possible to rule out a mutagenic mode of action by chemical D. Additional data on the chemical interactions of chemical D with macromolecules, and the nature of cytotoxic insults in target tissues and their relationship to tumor formation are needed.

APPENDIX E. NONLINEAR DOSE-RESPONSE: MARGIN OF EXPOSURE ANALYSIS

[To Be Developed]

APPENDIX F. DOSE-RESPONSE ASSESSMENT FOR A CARCINOGEN POSING HIGHER RISKS AFTER CHILDHOOD EXPOSURE

a. Introduction

Compound K is a carcinogenic to humans by all exposure routes. This conclusion is based on: (1) consistent epidemiologic evidence of a causal association between occupational exposure and the development of angiosarcoma, an extremely rare tumor; (2) suggestive epidemiological evidence that cancers of the brain, lung, and lymphopoietic system are associated with exposure; (3) consistent evidence of carcinogenicity in rats, mice, and hamsters via the oral and inhalation routes; (4) mutagenicity and DNA adduct formation by compound K and its metabolites in numerous *in vivo* and *in vitro* test systems; and (5) efficient absorption via all routes of exposure tested, followed by rapid distribution throughout the body.

Carcinogenicity involves genetic toxicity and is understood in some detail. Compound K is metabolized to a reactive metabolite, probably an epoxide, which is believed to be the ultimate carcinogenic metabolite. The reactive metabolite then binds to DNA, forming DNA adducts that, if not repaired, ultimately lead to mutations and tumor formation. Therefore, a linear extrapolation was used in the dose-response assessment. Because of uncertainty regarding exposure levels in the occupationally exposed cohorts, an inhalation unit risk of 2×10^{-6} per ug/m^3 was based on chronic inhalation studies in rats (not presented here).

Evidence has also been reported indicating increased sensitivity to early-life exposure. This case study shows how to use such evidence in a quantitative risk assessment. To focus on early-life exposures, the hazard assessment and dose-response assessment for chronic exposure (including derivation of the inhalation unit risk of 2×10^{-6} per ug/m^3) are not presented here.

b. Dose-response data for early-life exposure

A dose-rate study compared responses to different dosing regimens, in which rats inhaled compound K for 100 hours, starting at 13 weeks of age or 1 day of age (see table F-1). No effect

was observed for 100-hr exposures starting at 13 weeks, but 100-hr exposure starting at 1 day had a clear carcinogenic effect, causing both angiosarcomas and hepatomas.

Tumor incidences in the newborn rats were also compared with rats inhaling compound K for 52 weeks starting later in life (at 13 weeks) (see table F-2). Angiosarcoma incidence was comparable from 52-week exposure starting at 13 weeks and 5-week exposure starting at 1 day. Hepatoma incidence, however, was high after newborn exposure but virtually absent after chronic exposure starting later in life.

These data illustrate two phenomena that indicate higher cancer risks from childhood exposure: (1) high incidence of a tumor (angiosarcomas) also caused by adult exposure, and (2) occurrence of another tumor (hepatomas) not associated with adult exposure. The data suggest that risks from short-term, early-life exposure may not be reversible even in the absence of further exposure. The data do not, however, help us understand why early-life exposure poses greater risks. It could be that the metabolized dose is higher in newborns than in adults (either through more efficient metabolism, slower elimination, or a higher saturation point), alternatively, metabolized doses could be comparable in newborns and adults, but newborns could be biologically more sensitive to the same dose. Without understanding the mode of action early in life, we can nonetheless use these data to estimate the higher cancer risks caused by early-life exposure.

c. Dose conversion

Extensive pharmacokinetic studies show that the carcinogenic effects are caused by a metabolite and that metabolism becomes saturated below the tested doses. A PBPK model was fitted and validated (using independent data) to convert the experimental inhaled concentrations to equivalent human concentrations (see table F-3). This involved two steps: (1) convert experimental concentrations in air (ppm) to tissue concentrations in rat liver (mg metabolite per L liver), and (2) convert these tissue concentrations to equivalent human concentrations in air (ppm or mg/m^3). The inhalation unit risk for chronic adult exposure was derived using doses from this model.

Although the PBPK model was fitted using data on mature rats and adult human males,

dose estimates from this model were also used for dose-response modeling of tumors from early-life exposure. Similarly, although liver tissue concentrations were used as the dose metric in the PBPK model, this model was also used for angiosarcomas and angiomas at all sites (NTP guidance indicates that these tumors should be combined). Although the ideal would be to have pharmacokinetic information on various tissue concentrations in children, these studies have not been conducted. The lack of this information introduces some uncertainty into the results. Use of the PBPK model reflects a conscious decision that a credible dose-response model would be based on saturable metabolism and not on administered concentrations alone.

Although it is standard practice to calculate lifetime average daily doses for carcinogens (U.S. EPA, 1992), a different approach may be appropriate when considering effects of childhood exposures if children are more sensitive than adults. Specifically, it may not be appropriate to average childhood exposures over a full lifetime, since that implies that childhood exposure is equivalent to full-life exposure at a lower rate. Consequently, the dose estimates from the PBPK model are not averaged over a lifetime. Instead, the average dose during the early-life period (in this experiment, 5 weeks) is used. That is, the administered concentration is reduced to reflect intermittent exposure of 4 hr/d, 5 d/wk, but there is no further reduction by the ratio of the early-life period (5 wk) to a lifetime. This childhood exposure estimate is applied to the childhood-specific unit risk estimate calculated below. (If a unit risk estimate could not be calculated from the early-life experiments and the adult unit risk estimate were used instead, the adult unit risk would be adjusted for children as discussed in section 3.5.2.)

d. Analysis in the range of observation

In the range of observation, incidences of angiosarcomas or hepatomas (from table F-1) are modeled separately as functions of equivalent human concentration based on metabolized dose (from table F-3) using a quantal polynomial model of the form

$$p(d) = 1 - \exp(-q_1 d - \ldots - q_k d^k),\ q_1, \ldots, q_k \geq 0$$

The resulting *points of departure* are LEC_{10} = 36 ppm for angiosarcomas and LEC_{10} = 33 ppm for hepatomas. Converting these to units of ug/m^3 (for this compound, 1 ppm = 2600 ug/m^3) yields $LEC_{10} = 9.4 \times 10^4$ ug/m^3 for angiosarcomas and $LEC_{10} = 8.6 \times 10^4$ ug/m^3 for hepatomas.

e. Extrapolation to lower doses

The available mechanistic information, which indicates a reactive metabolite that binds to DNA and forms DNA adducts that ultimately lead to mutations and tumor formation, supports linear extrapolation to lower doses. Linear extrapolation follows the line from the point of departure to the origin (zero dose, zero excess risk). The slope of this line is $0.10/LEC_{10}$. Accordingly, the unit risk estimates are 1.1×10^{-6} per ug/m^3 for angiosarcomas and 1.2×10^{-6} per ug/m^3 for hepatomas.

f. Combining unit risk estimates for multiple tumor types

To obtain an estimate of overall cancer risk, the unit risks for the induced tumor types are combined. In the absence of individual animal pathology data, a neutral assumption is that the tumor types are independent. In this case, the induction of angiosarcomas but not hepatomas by later-life exposure suggests that these tumor types are caused by different modes of action and may be independent. Under an assumption of independence, the combined unit risk is

$$1.1 \times 10^{-6} + 1.2 \times 10^{-6} - (1.1 \times 10^{-6} \times 1.2 \times 10^{-6}) = 2.3 \times 10^{-6} \text{ per ug/m}^3$$

g. Strengths and limitations of the data

Although the data on newborn animals come from one rat strain over a limited range of inhalation concentrations and there are no epidemiologic studies of children exposed to this compound, the animal results indicate a potential for an increased susceptibility to tumors if children are exposed. Another limitation is that individual animal data are not available to determine whether animals with angiosarcomas are more likely to have hepatomas. Without these data, an assumption of independence was made when combining unit risks across multiple tumor sites.

The conversion used in this assessment to obtain the human continuous exposure concentrations in ppm from the corresponding human dose metric in mg/L was a linear one. This conversion methods seems simplistic given the complexity of the human body. This conversion may be not be unreasonable, however, because this compound is rapidly and efficiently absorbed, converted to water-soluble metabolites, and excreted.

h. Application to less-than-lifetime exposure scenarios

Two observations about the early-life studies have implications for how this assessment would be applied to less-than-lifetime exposure scenarios, particularly during childhood.

1. The exposure period in the early-life experiment (weeks 1-5) does not overlap that of the chronic experiments (weeks 14-65) used to estimate the inhalation unit risk for chronic adult exposure. Therefore, the full lifetime cancer risk can be approximated by adding risks from these nonoverlapping exposure periods.
2. Because the effects of early-life exposure are different from effects of later exposures, it would not be appropriate to prorate childhood exposures as if they were received at a proportionately lower rate over a full lifetime.

These observations imply that the potential for increased sensitivity to childhood exposure is not reflected in the unit risk estimated from later-life exposures. The following examples illustrate how to combine early-life and later-life unit risk estimates.

Example 1. Full lifetime exposure (birth through death) to 1 ug/m^3

The total risk is made up of two components, an early-life risk and a later-life risk.

Risk from early-life exposure: (2.3×10^{-6} per ug/m^3) x (1 ug/m^3) = 2.3×10^{-6}

Risk from later-life exposure: (2×10^{-6} per ug/m^3) x (1 ug/m^3) = 2×10^{-6}

Total risk: 4.3×10^{-6}

Example 2. Exposure to 2 ug/m^3 from ages 30-60

Because exposure begins at age 30, there is no early-life component. The later-life component is prorated as a duration of 30 years over an assumed lifespan of 70 years.

Risk from early-life exposure: Not applicable

Risk from later-life exposure: (2×10^{-6} per ug/m^3) x (2 ug/m^3) x (30/70) = 1.7×10^{-6}

Total risk: 1.7×10^{-6}

Example 3. Exposure to 5 ug/m^3 from ages 0-10

Here there is an early-life component that is not prorated. The later-life component is, however, prorated as 10 out of 70 years.

Risk from early-life exposure: $(2.3 \times 10^{-6}$ per ug/m^3) x (5 ug/m^3) = 1.2×10^{-5}

Risk from later-life exposure: $(2 \times 10^{-6}$ per ug/m^3) x (5 ug/m^3) x (10/70) = 1.4×10^{-6}

Total risk: 1.3×10^{-5}

Table F-1. Comparison of tumor incidences in male and female Sprague-Dawley rats from 100-hr inhalation exposures to newborn and mature rats

	Angiosarcomas and angiomas (all sites)			Liver hepatomas		
Inhaled concentration (ppm)	Control[a]	6000 ppm	10,000 ppm	Control[a]	6000 ppm	10,000 ppm
4 hr/d, 5 d/wk, 5 wk, starting at age 13 wk	1/277	3/120	2/118	0/277	0/120	1/118
1 hr/d, 4 d/wk, 25 wk, starting at age 13 wk	1/277	5/118	4/119	0/277	0/118	0/119
4 hr/d, 1 d/wk, 25 wk, starting at age 13 wk	1/277	4/120	4/119	0/277	2/120	0/119
4 hr/d, 5 d/wk, 5 wk, starting at age 1 day	1/277	20/42	18/44	0/277	20/42	20/44

[a]One control group served for all exposure patterns

Table F-2. Comparison of tumor incidences in male and female Sprague-Dawley rats from 5-wk newborn exposure and 52-wk later-life exposure

	Angiosarcomas and angiomas (all sites)			Liver hepatomas		
Inhaled concentration (ppm)	Control	6000 ppm	10,000 ppm	Control	6000 ppm	10,000 ppm
4 hr/d, 5 d/wk, 52 wk, starting at age 13 wk	2/58	22/59	13/60	0/58	1/59	1/60
4 hr/d, 5 d/wk, 5 wk, starting at age 1 day[a]	1/277	20/42	18/44	0/277	20/42	20/44

[a]Repeated from table F-1

Table F-3. Results of PBPK modeling			
Inhaled concentration (ppm)	Control	6000 ppm	10,000 ppm
Internal dose of metabolite (mg metabolite / L liver)	0	395	404
Equivalent continuous human inhaled concentration (ppm)	0	251	257

APPENDIX G. RESPONSE TO COMMENTS ON OTHER SCIENCE ISSUES

1 [To Be Developed]